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RESEARCH ARTICLE

Genetic effects of habitat fragmentation and population isolation on *Etheostoma raneyi* (Percidae)

Ken A. Sterling · David H. Reed · Brice P. Noonan · Melvin L. Warren Jr.

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Abstract The use of genetic methods to quantify the effects of anthropogenic habitat fragmentation on population structure has become increasingly common. However, in today's highly fragmented habitats, researchers have sometimes concluded that populations are currently genetically isolated due to habitat fragmentation without testing the possibility that populations were genetically isolated before European settlement. *Etheostoma raneyi* is a benthic headwater fish restricted to river drainages in northern Mississippi, USA, that has a suite of adaptive traits that correlate with poor dispersal ability. Aquatic habitat within this area has been extensively modified, primarily by flood-control projects, and populations in headwater streams have possibly become genetically isolated from one another. We used microsatellite markers to quantify genetic structure as well as contemporary and historical gene flow across the range of the species. Results indicated that genetically distinct populations exist in each headwater stream analyzed, current gene flow rates are lower than historical rates, most genetic variation is partitioned among populations, and populations in the Yocona River drainage show lower levels of genetic diversity than populations in the Tallahatchie River drainage and other *Etheostoma* species. All populations have negative F_{IS} scores, of which roughly half are significant relative to Hardy–Weinberg expectations, perhaps due to small population sizes. We conclude that anthropogenic habitat alteration and fragmentation has had a profoundly negative impact on the species by isolating *E. raneyi* within headwater stream reaches. Further research is needed to inform conservation strategies, but populations in the Yocona River drainage are in dire need of management action. Carefully planned human-mediated dispersal and habitat restoration should be explored as management options across the range of the species.

Keywords *Etheostoma* · Habitat fragmentation · Genetic structure · Barrier · Population isolation · Excess heterozygosity

D. H. Reed—Deceased.

K. A. Sterling · B. P. Noonan
Department of Biology, University of Mississippi,
P.O. Box 1848, University, MS 38677, USA

Present Address:
K. A. Sterling (✉)
Department of Wildland Resources, Utah State University,
5230 Old Main Hill, Logan, UT 84322, USA
e-mail: kensterling39@gmail.com

D. H. Reed
Department of Biology, University of Louisville, Louisville,
KY 40292, USA

M. L. Warren Jr.
USDA Forest Service, Southern Research Station,
Center for Bottomland Hardwoods Research, 1000 Front Street,
Oxford, MS 38655, USA

Introduction

For freshwater stream fishes in the southeastern United States, habitat destruction and fragmentation have led to the genetic isolation of populations in numerous species (Jelks et al. 2008) and is a leading conservation concern (Warren et al. 2000; Kuhajda et al. 2008). Reductions in habitat quality, small population size, and lack of gene flow are predicted to result in declines and extirpations of populations (Reed 2008). The Yazoo darter (*Etheostoma raneyi*) is endemic to the Yocona River (Yocona R. hereafter) and

Little Tallahatchie River (Tallahatchie R. hereafter) drainages of the upper Yazoo River basin in north-central Mississippi, USA (Thompson and Muncy 1986; Suttkus et al. 1994; Johnston and Haag 1996) (Fig. 1). This restricted range, combined with habitat degradation and fragmentation within that range, puts the Yazoo darter at risk of extinction. The species is classified as vulnerable by the Southeastern Fishes Council (Warren et al. 2000) and American Fisheries Society (Jelks et al. 2008) and as globally imperiled by the Nature Conservancy (NatureServe 2011).

The range of the Yazoo darter lies within the Northern Hilly Gulf Coastal Plain Ecoregion of north-central Mississippi which consists of low rolling hills 80–180 m in elevation (Chapman et al. 2004). The region has experienced significant anthropogenic habitat alteration, coinciding with European settlement, over the past 150–180 years. During this period, forests were removed and land converted to agricultural use which led to widespread and dramatic erosion, filling stream valleys with sediment and exacerbating flooding problems (Cooper and Knight 1991; Shields et al. 1994). Localized efforts to prevent flooding and reclaim valley lands by straightening and channelizing streams were met with little success (Shields et al. 1994). The Great Flood of 1927 affected seven states, including Mississippi, and prompted the federal government to action. Within the range of the Yazoo

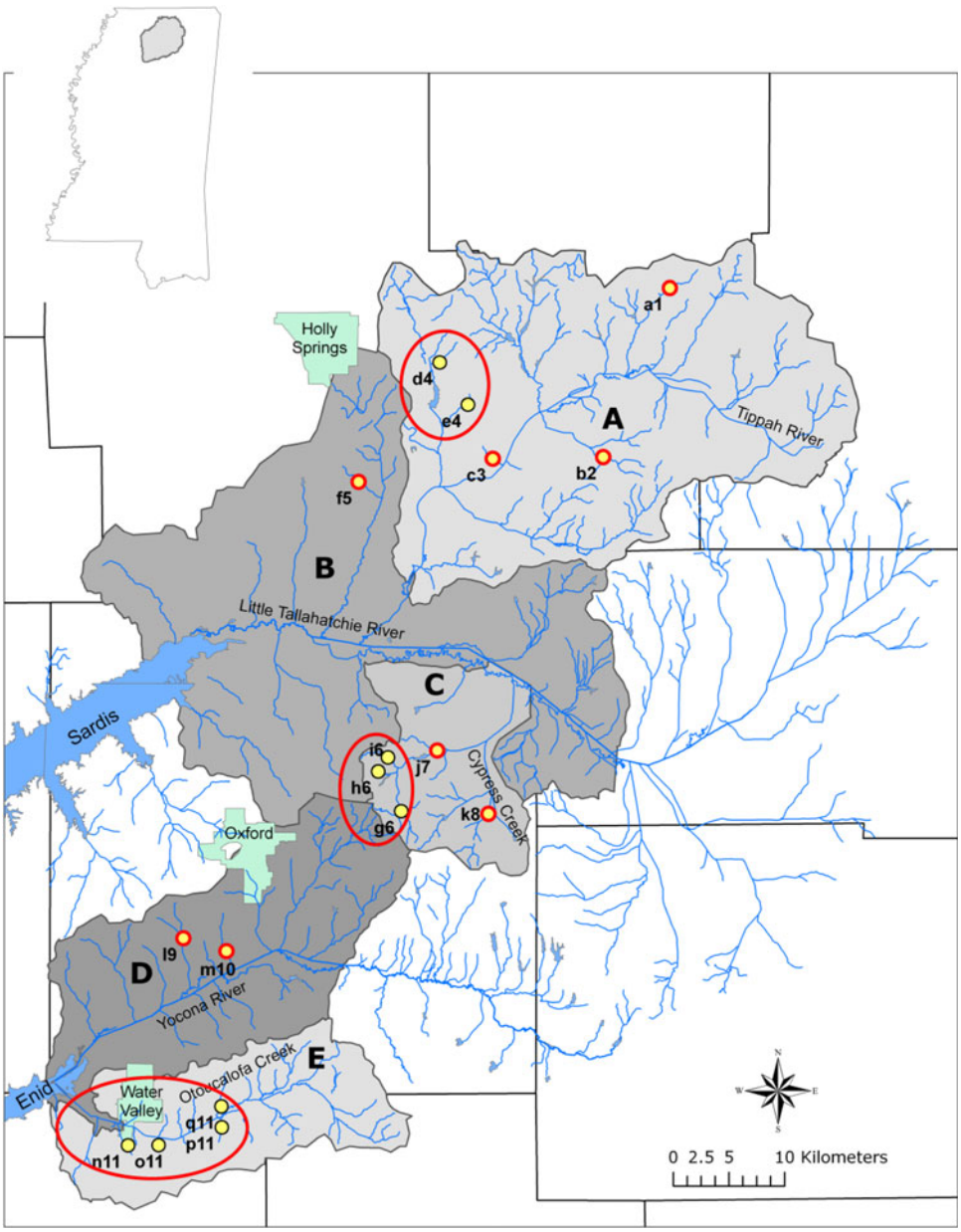

Fig. 1 Major drainages, watershed units, and cities within the range of the Yazoo darter (*shaded area*) in north-central Mississippi. Numbered *yellow circles* and *red circles* correspond to DNA tissue sample sites and genetic clusters (GENELAND), respectively (Table 1). Tallahatchie R. watershed units are outlined and lettered as: *A* Tippah River Unit; *B* Tallahatchie River Tributaries Unit; *C* Cypress Creek Unit. Yocona R. watershed units are outlined and lettered as: *D* Yocona River Unit; *E* Otoucalofa Creek Unit

darter, large (~40,400 ha) flood control impoundments were constructed on each of the two major drainages, the Yocona R. and Tallahatchie R., extensive stream reaches were straightened and channelized, and hundreds of small impoundments were built in headwater streams. This altered stream gradients which resulted in stream incisement and headcutting in nearly all headwater streams (Shields et al. 1998). Channelized and incised streams tend to be shallow, sandy, homogeneous, turbid, and unstable with flashy flows (Shields et al. 1994, 1998; Simon and Darby 1997; Adams et al. 2004).

Yazoo darters are small (<65 mm SL, standard length), benthic insectivores living up to 3 years, though most individuals do not survive their first year (Johnston and Haag 1996). They are restricted to perennial, small, headwater streams which are often spring-fed (Thompson and Muncy 1986; Suttkus et al. 1994). Spawning season is dependent on water temperatures, usually commencing in early March and ending mid-May (Suttkus et al. 1994; Johnston and Haag 1996). Yazoo darters have relatively low fecundity, attaching their eggs singly to available substrate, and newly hatched larvae are free-swimming (Johnston and Haag 1996; unpubl. data). This suite of traits is adaptive for headwater stream fishes and is associated with reduced gene flow among streams (Turner and Trexler 1998).

Genetic methods are an effective way to assess the impact of habitat fragmentation on dispersal and resultant population structure in fishes (Powers et al. 2004; George et al. 2008; Skalski et al. 2008). However, most studies of southeastern stream fishes to date have not compared observed patterns of genetic structure with historic estimates of dispersal (but see Fluker et al. 2010). In highly fragmented stream systems, this may lead workers to conclude that contemporary population structure is a result of habitat fragmentation without considering the possibility that populations were always fragmented (Chiucchi and Gibbs 2010; Reed et al. 2011), which is a distinct possibility in headwater stream fishes (Turner and Trexler 1998; Turner and Robison 2006). Our goal here is to quantify contemporary population genetic structure, and to contrast our results with historic and contemporary estimates of dispersal between populations across the range of the Yazoo darter.

Materials and methods

Sample collection and site locations

Tissue samples were collected by taking small fin clips from 212 individual Yazoo darters at 17 sites (Table 1; Fig. 1). With the exception of voucher specimens from streams where Yazoo darters had not been sampled before, individuals were released alive and tissue samples were immediately stored in 95% ethanol. Fish were captured using single-pass backpack electrofishing, seines, and dip nets. Yazoo darters were collected up- and downstream of two small impoundments (Chewalla and Puskus lakes) and in tributaries separated by channelized stream reaches which were hypothesized to be barriers to dispersal. Tissue samples were also collected across the range of the species including all major stream systems. Within the Yocona R. drainage, samples were collected from two sites (l9, m10) in the Yocona R. watershed and four sites (n11, o11, p11, q11) within the Otoucalofa Creek watershed. Within the Tallahatchie R. drainage, samples were collected from one site (f5) in the Big Spring Creek watershed, five sites (a1, b2, c3, d4, e4) within the Tippah River watershed, and five sites (g6, h6, i6, j7, k8) within the Cypress Creek watershed. This allowed for characterization of population structure within and between watersheds of each river drainage (Fig. 1).

Microsatellite amplification and allele scoring

Nuclear DNA was extracted using standard phenol–chloroform methods (Taggart et al. 1992) and target sequences were amplified by PCR using 10 microsatellite primers developed for other species of *Etheostoma*: Esc 26b, Esc 18, Esc 187 (*Etheostoma scotti*) (Gabel et al. 2008); Etsp 224, Etsp 227, Etsp 208, Etsp 219 (*Etheostoma spectabile*) (Hudman et al. 2008); Eche 010 (*Etheostoma chermocki*) (Khudamrongsawat et al. 2007); and EosD 116, EosD 108 (*Etheostoma osburni* and *E. variatum*) (Switzer et al. 2008). PCR reaction volume (11.5 µl) contained 10× reaction buffer (Genesee Scientific San Diego, CA), 50 mM $MgCl_2$ (Genesee Scientific San Diego, CA), 160 µM each dNTP, 1 µM each primer, and 1 U *Taq* polymerase (Apex Taq, Genesee Scientific San Diego, CA). The PCR cycle was 94°C/1 min followed by 20 cycles of: 94°C/30 s, 60°C/25 s decreasing every cycle by 0.3C°, 72°C/40 s; then 8 cycles of: 94°C/30 s, 56°C/25 s, 72°C/40 s and a final extension of 72°C/30 min. PCR product was genotyped on an ABI 3730 sequencer (Applied Biosystems) and fragment sizes scored using Peak Scanner software (Applied Biosystems), manually checking all scores. All homozygous individuals and subsets of heterozygous individuals were amplified and scored for each locus at least twice to check for consistency.

Population differentiation and genetic analysis

The program GENELAND ver. 3.2.4 was used (Guillot et al. 2005; Guillot and Santos 2009) to determine the number (K) of genetically distinct population clusters and their membership for further analyses. Because population structure was expected to be largely due to recent habitat

Table 1 Site ID (corresponding to Fig. 1), population assignment, sample size, watershed unit, stream and location of each Yazoo darter tissue sample site used for DNA analysis

Site ID	Population	*n*	Watershed unit	Stream	Latitude	Longitude
a	1	13	Tippah River	Yellow Rabbit Creek	34.819	89.105
b	2	21	Tippah River	South Chilli Creek	34.682	89.172
c	3	11	Tippah River	Tippah River Tributary	34.708	89.255
d	4	9	Tippah River	Chewalla Creek Tributary upstream of dam	34.76	89.332
e	4	6	Tippah River	Chewalla Creek Tributary downstream of dam	34.725	89.305
f	5	22	Tallahatchie R.	Big Spring Creek Tributary	34.663	89.412
g	6	5	Cypress Creek	Puskus Creek upstream of dam	34.395	89.372
h	6	11	Cypress Creek	Puskus Creek upstream of dam	34.428	89.394
i	6	10	Cypress Creek	Puskus Creek upstream of dam	34.431	89.375
j	7	21	Cypress Creek	Puskus Creek downstream of dam	34.445	89.336
k	8	19	Cypress Creek	Cypress Creek	34.393	89.286
l	9	12	Yocona R.	Taylor Creek Tributary	34.123	89.641
m	10	11	Yocona R.	Morris Creek	34.282	89.543
n	11	7	Otoucalofa Creek	Johnson Creek	34.123	89.641
o	11	13	Otoucalofa Creek	Otoucalofa Creek Tributary	34.125	89.61
p	11	5	Otoucalofa Creek	Gordon Branch	34.14	89.549
q	11	16	Otoucalofa Creek	Mill Creek	34.166	89.52

alteration of landscape features such as channelized stream reaches, the spatial and correlated model options were chosen as suggested by Guillot and Santos (2009). The correlated model in GENELAND uses a Bayesian clustering algorithm and spatial data (georeferenced coordinates) to assign individuals probabilistically to clusters or populations without a priori knowledge of population units accounting for null alleles and isolation by distance. The model assumes populations are in Hardy–Weinberg equilibrium, loci are not linked, and genotypes are spatially correlated. Computations are carried out through the Markov Chain Monte Carlo (MCMC) method. Ten independent runs of the program were used, allowing K to vary from one to seventeen clusters and 10^6 MCMC iterations to check for consistent convergence on a solution, using the modal value of these runs to infer the number of clusters. The resulting populations were tested for significant differentiation (genic and pairwise F_{ST}), gametic equilibrium, and departure from Hardy–Weinberg equilibrium as described later.

STRUCTURE ver. 2.3.3 (Pritchard et al. 2000), another clustering program, was used to check for consistency with GENELAND results. Six independent runs of 300,000 replicates and 30,000 burn-in cycles were used, varying the number of populations from $K = 1$–13. The admixture model was used which allows individuals in a given population to have mixed ancestry. The "Locprior" (Hubisz et al. 2009) option was chosen which uses a priori sampling location data (georeferenced coordinates). The correlated allele frequency option (Falush et al. 2003) was used which, similar to GENELAND, assumes that allele frequencies are correlated among populations due to dispersal or shared ancestry. The rate of drift (F_k) was allowed to assume a different value for each population, and K was estimated using the ad hoc summary statistic ΔK (Evanno et al. 2005).

All individuals were grouped into eleven populations as determined by GENELAND for the following analyses. FSTAT ver. 2.9.3.2 (Goudet 2001) was used to test for Hardy–Weinberg equilibrium within populations using the heterozygote excess method (15,000 permutations), to estimate F_{IS} per population and locus, to calculate unbiased gene diversity (H_S) (Nei 1987) and to calculate allelic richness (A_R) per population using rarefaction. The permutation test in FSTAT (15,000 permutations) was used to test differences in A_R, H_O, and H_S between the Yocona R. and Tallahatchie R. drainages. GENEPOP (Raymond and Rousset 1995; Rousset 2008) was used to estimate frequency of null alleles per locus and to test for gametic disequilibrium and population (genic) differentiation. To calculate observed heterozygosity (H_O), expected heterozygosity (H_E), pairwise F_{ST} between populations and to test for significance of F_{ST} estimates, ARLEQUIN ver. 3.5.1.2 was used (Excoffier et al. 2005). A modified false discovery rate (FDR) (Benjamini and Yekutieli 2001; Narum 2006) correction was used to prevent Type I errors whenever multiple tests were performed. To test for isolation by distance, a Mantel test (Mantel 1967; Bohonak 2002) of linearized F_{ST} versus geographic distance was used as implemented in the program IBD (Jensen et al. 2005). Geographic and F_{ST} distance values

were tested for isolation by distance across all populations and across all populations within the Yocona R. drainage and the Tallahatchie R. drainage, respectively.

To further investigate population structure, analysis of molecular variance (AMOVA) as implemented in ARLEQUIN (Excoffier et al. 1992) was used. This method performs a standard analysis of variance (ANOVA) where the total variance is partitioned in covariance components due to variation among individuals, among populations, and among groups of populations. Fixation indices are calculated with the covariance components among groups of populations (F_{CT}), among populations within groups (F_{SC}), and among individuals within populations (F_{IS}). Significance levels were obtained with 30,000 permutations. A hierarchical design (Fig. 1) was used to group populations as defined by GENELAND. To examine structure between the two major river drainages, populations within the Yocona R. and Tallahatchie R. drainages were grouped. To examine structure between watersheds within the Yocona R. drainage, the Yocona R. watershed populations were grouped and compared to the population in the Otoucalofa Creek watershed. Within the Tallahatchie R. drainage, populations in the Cypress Creek watershed, the Tippah River watershed, and the Big Spring Creek watershed were grouped for comparison. To examine structure within a watershed, the four populations within the Tippah River watershed were compared.

Estimating contemporary and historical gene flow

MIGRATE ver. 3.2.1 (Beerli and Felsenstein 1999) was used to estimate historical levels of migration and BAYESASS ver. 1.3 (Wilson and Rannala 2003) was used to estimate contemporary levels of migration. MIGRATE uses a coalescent approach to estimate mutation-scaled migration rates (M) for each population analyzed over about the last $4N_e$ generations (Beerli 2010), a period of time estimated to range from between 100 and 500 years depending on generation time. Generation time for Yazoo darters was not calculated because of lack of information to do so. However, a generation time of 1.0–1.5 years was estimated because most fish do not survive their first year and few survive to their third year (Johnston and Haag 1996). Computations were carried out through the MCMC method. The major assumptions of the model are that all populations exchanging genes have been sampled, population sizes and migrations rates have not changed over time, mating is random, loci are neutral, and recombination occurs at low to moderate levels. The maximum likelihood option, the Brownian motion mutation model, and the matrix migration model were used. Fifteen short chains were run, sampling every 100 generations until 500 genealogies were recorded from 50,000 genealogies sampled after a burn-in of 30,000, and then four long chains were run sampling every 400 generations until 20,000 genealogies were recorded from 8×10^6 genealogies sampled after a burn-in of 30,000. The "summarize over all chains" option was chosen because this is recommended for difficult data sets (Beerli 2010). This option combines the results of long chains to estimate parameters. Three independent runs were performed to ensure that the program was producing consistent parameter estimates.

BAYESASS is another Bayesian inference MCMC program which estimates asymmetric migration over the last two to three generations (Wilson and Rannala 2003) or about 3–5 years using 1.0–1.5 years per generation for Yazoo darters (Johnston and Haag 1996). Unlike MIGRATE, deviations from Hardy–Weinberg expectations do not violate the assumptions of the model. The program was run for 3×10^7 iterations, sampling every 2,000 iterations with a burn-in of 3×10^6 iterations. Delta, a parameter that defines the maximum value a parameter can change in each iteration, was set to 0.1, 0.2, and 0.1 for allele frequency, migration rate, and inbreeding respectively. Ten independent runs were performed, each with a different initial seed value, and then a Bayesian deviance measure was used which determined the run that best fit the data (Spiegelhalter 2002). Using the initial seed value from the best run, the number of iterations was increased to 9×10^7 for a final run with a burn-in of 1×10^7 (Chiucchi and Gibbs 2010). Reported results are from this final run. In addition, mean ancestry values (q) were also used from the six independent STRUCTURE simulations where $K = 11$ to identify migrants (Estes-Zumpf et al. 2010). Individuals were considered migrants if they had >70% ancestry from a population other than that from which they were sampled.

Separate analyses were performed in MIGRATE and BAYESASS for the Yocona R. and Tallahatchie R. populations based on results from Powers and Warren (2009) indicating that these populations are genetically isolated with respect to one another. Because we were interested in dispersal across watersheds within river drainages and across tributaries within watersheds, and to reduce the number of pairwise comparisons, populations were grouped as described previously for AMOVA. Individuals sampled upstream of Puskus Lake and Chewalla Lake were not included in these groups because they are currently isolated from other individuals downstream that are included in the analysis.

Results

Cluster analyses

Each independent GENELAND run grouped Yazoo darters into eleven clusters of identical composition (Table 1;

Fig. 1). Within the Yocona R. drainage, the four sites in different tributaries of Otoucalofa Creek were grouped together (n11, o11, p11, q11). However, the two sites (l9, m10) in neighboring tributaries of the Yocona R. were genetically distinct though the two tributaries are only about 5 km apart. Within the Tallahatchie R. drainage, the three sites upstream of Puskus Lake in Puskus Creek (i6, h6, g6) and two of its tributaries were grouped together and were distinct from the single site downstream of Puskus Lake (j7). Within Chewalla Creek, the sites upstream and downstream of Chewalla Lake were not distinct, forming a single cluster (d4, e4). All three sampling sites (c3, b2, a1) within the Tippah River watershed contained distinct genetic clusters. Posterior probabilities of population membership assigned all individuals within a sampling site to the same cluster.

Post hoc analysis of STRUCTURE output (Evanno et al. 2005) supported seven rather than eleven clusters. This clustering was consistent with GENELAND results in that the additional clusters found by GENELAND were the result of subdivision of those recovered by STRUCTURE. For the STRUCTURE runs where $K = 11$, cluster assignment of individuals was identical to GENELAND results.

Genetic diversity and population structure

Null alleles were estimated to be <0.05% for any given locus. Two pairs of loci of 45 pairs tested (adjusted $\alpha = 0.0114$) were significantly out of gametic equilibrium overall after correcting for multiple tests (Etsp 219 and Etsp 224, $p \leq 0.0001$; Esc 26b and Esc 187, $p \leq 0.0001$). However, tests of linkage disequilibrium within each population showed that, after correction for multiple tests within each population, only Etsp 219 and Etsp 224 remained significantly out of equilibrium, and only for the Otoucalofa Creek population. Based on these results we assume that all loci are unlinked.

Our tests for heterozygosity excess show that five populations were significantly out of Hardy–Weinberg equilibrium after correction for multiple tests (adjusted $\alpha = 0.016$; Morris (m10), $p \leq 0.0005$; Big Spring (f5), $p \leq 0.002$; Chewalla (d4, e4), $p \leq 0.003$; Tippah River tributary (c3), $p \leq 0.001$; and Cypress (k8), $p \leq 0.0005$) and three others (Yellow Rabbit (a13), $p \leq 0.08$; Chilli (b2), $p \leq 0.07$; and Puskus downstream of Puskus Lake (j7), $p \leq 0.02$) are marginally significant. F_{IS} scores for all populations were negative and ranged from −0.326 to −0.013. The number of alleles per locus ranged from six to 51. Allelic richness ($p \leq 0.007$), observed heterozygosity ($p \leq 0.007$), and unbiased gene diversity ($p \leq 0.006$) were all significantly higher in the Tallahatchie R. drainage than in the Yocona R. drainage. Likewise, estimates of mean allelic richness of four other species of *Etheostoma* (Edberg 2009; Fluker et al. 2010) are higher than in Yazoo darters (Table 2), though confidence intervals overlap with those of Yazoo darters from the Tallahatchie R. drainage, but not the Yocona R. drainage. Mean observed and expected heterozygosity of nine other species of *Etheostoma* (Tonnis 2006; Beneteau et al. 2007; Khudamrongsawat et al. 2007; Switzer et al. 2008; Gabel et al. 2008; Hudman et al. 2008; Haponski et al. 2009; Fluker et al. 2010) show that for mean observed heterozygosity, confidence intervals overlap in all cases. However, mean expected heterozygosity was marginally lower for the Yocona R. drainage than the Tallahatchie R. drainage and other *Etheostoma* species and similar between other *Etheostoma* species and the Tallahatchie R. drainage with overlapping confidence intervals (Table 2). We did not test for differences in genetic diversity between other *Etheostoma* species and Yazoo darters because we have data for only a few other *Etheostoma* species, sampling methods differ among studies, sample sizes are small for these species, and because many of these species are of conservation concern with small population sizes: five of nine species used for comparing heterozygosity are considered Endangered,

Table 2 Mean (±95% CI) allelic richness (A_R), gene diversity (H_S), observed heterozygosity (H_O), and expected heterozygosity (H_E) for Yazoo darters in the Yocona R. and Tallahatchie R. drainages with a comparison of mean (±95% CI) A_R, H_O, and H_E of other species of *Etheostoma* darters

	A_R	H_S	H_O	H_E
Yocona R. drainage				
Mean	4.66	0.585	0.676	0.608
±95% CI	0.92	0.1	0.1	0.09
n	30	30	29	29
Tallahatchie R. drainage				
Mean	6.89	0.745	0.822	0.766
±95% CI	0.59	0.05	0.05	0.05
n	80	80	78	78
Etheostoma				
Mean	7.63	–	0.735	0.779
±95% CI	0.85	–	0.1	0.08
n	34	–	96	96

Table 3 Pairwise F_{ST} scores below diagonal, pairwise geographic distances (km) above diagonal; letter and number codes for populations are cross-referenced with Table 1 and Figure 1

	n, o, p, q11	m10	l9	f5	d, e4	a1	c3	b2	k8	j7	g, h, i6
n, o, p, q11	0	34.12	33.75	158.3	171.2	195.1	174.1	182.4	172	171.75	176.05
m10	0.219	0	11.9	166.65	179.9	203.3	182.06	191.05	180.9	180.15	184.5
l9	0.199	0.17	0	166.1	179.34	202	181.6	190.8	180.25	179.75	184.05
f5	0.28	0.267	0.225	0	46.7	68.3	48.2	57	46.4	45.6	50.1
d, e4	0.248	0.229	0.172	0.104	0	39	19.3	28.9	53.1	52	56.75
a1	0.24	0.201	0.165	0.091	0.04	0	20.9	25.5	74.3	74	78.5
c3	0.254	0.228	0.187	0.102	0.063	0.034	0	10.2	54.3	53.7	58.25
b2	0.287	0.276	0.221	0.063	0.086	0.08	0.109	0	63.8	63.5	68
k8	0.293	0.285	0.262	0.103	0.095	0.097	0.124	0.114	0	9.5	13.8
j7	0.271	0.26	0.211	0.069	0.079	0.078	0.085	0.07	0.051	0	4.3
g, h, i6	0.281	0.265	0.223	0.094	0.01	0.083	0.117	0.088	0.064	0.047	0

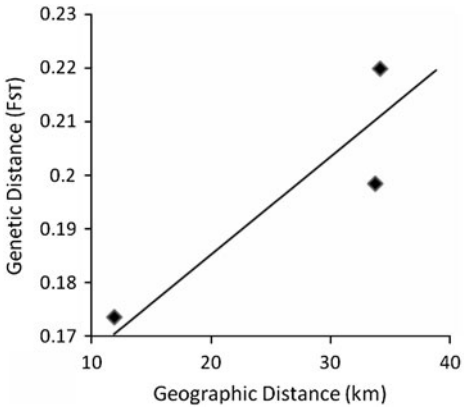

Fig. 2 Plot of Mantel test for isolation by distance across all populations in the Yocona R. drainage (r = 0.89, $p < 0.0001$)

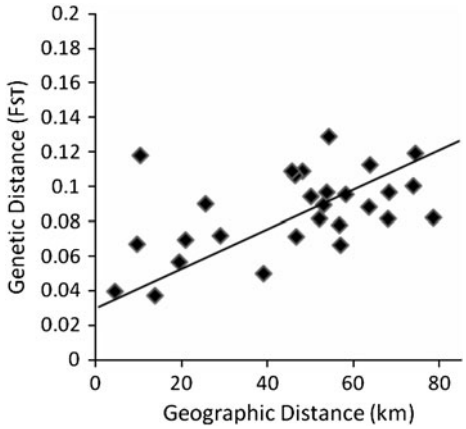

Fig. 3 Plot of Mantel test for isolation by distance across all populations in the Tallahatchie R. drainage (r = 0.5, $p = 0.015$)

Threatened, or Vulnerable, and one of four species used for comparing allelic richness was classified as Endangered (Warren et al. 2000).

Pairwise F_{ST} values ranged from 0.034 to 0.123 within the Tallahatchie R. drainage, from 0.17 to 0.21 within the Yocona R. drainage and from 0.17 to 0.29 between the Yocona R. and Tallahatchie R. drainages (Table 3). All pairwise F_{ST} values were significant (adjusted $\alpha = 0.0109$, $p \leq 0.0001$), and population differentiation was significant (adjusted $\alpha = 0.0109$, $X^2 = \infty$, $p \leq 0.0001$) for all pairs. The test of isolation by distance was significant over all populations (r = 0.86, $p = 0.0002$), within the Yocona R. drainage (r = 0.89, $p < 0.0001$) (Fig. 2), and within the Tallahatchie R. drainage (r = 0.50, $p = 0.015$) (Fig. 3). When comparing genetic variation (AMOVA, Table 4), most of the genetic variation occured between the Yocona R. and Tallahatchie R. drainages ($F_{CT} = 0.137$, df = 1, $p < 0.007$), but a significant amount of variation also occured among populations within each drainage ($F_{SC} = 0.097$, df = 8, $p < 0.0001$). No significant variation occured among individuals within populations, but there is a significant amount of variation within individuals ($F_{IT} = 0.85$, df = 196, $p < 0.0001$). Comparisons among groups of populations within watersheds within each drainage show that most of the significant genetic variation is among populations within watersheds (Yocona R.: $F_{SC} = 0.172$, df = 1, $p < 0.0001$; Tallahatchie R.: $F_{SC} = 0.063$, df = 5, $p < 0.0001$) and that lower (insignificant) amounts of among-watershed variation occur in the Yocona R. drainage ($F_{CT} = 0.047$, df = 1, $p < 0.33$), but marginally significant amounts of among-watershed variation exist in the Tallahatchie R. drainage ($F_{CT} = 0.032$, df = 2, $p < 0.013$). There is no significant variation among individuals within populations or within individuals of any watershed. Comparisons among

Table 4 Analysis of molecular variance (AMOVA) between groups of populations from the Yocona R. and Tallahatchie R. drainages, between groups of populations from watersheds within each drainage, and between populations within the Tippah River watershed

Source of variance	df	Sum of Squares	Variance component	Variance (%)	Fixation index (*p*)
AMOVA between the Yocona R. drainage and L.T.R. drainage					
Among groups	1	126.87	0.588 Va	13.68	F_{CT} (0.007)
Among populations within groups	8	145.01	0.417 Vb	9.69	F_{SC} (<0.0001)
Among individuals within populations	186	550.18	−0.337 Vc	−7.84	F_{IS} (1.0)
Within individuals	196	712	3.63 Vd	84.47	F_{IT} (<0.0001)
AMOVA between the Yocona R. and Otoucalofa Creek watersheds					
Among groups	1	37.58	0.164 Va	4.69	F_{CT} (0.33)
Among populations within groups	1	15.68	0.575 Vb	16.42	F_{SC} (<0.0001)
Among individuals within populations	61	151.97	−0.270 Vc	−7.71	F_{IS} (1.0)
Within individuals	64	194	3.03 Vd	86.61	F_{IT} (0.07)
AMOVA between the Tippah River, Big Spring Creek, and Cypress Creek watersheds					
Among groups	2	48.22	0.126 Va	3.2	F_{CT} (0.013)
Among populations within groups	5	57.09	0.24 Vb	6.12	F_{SC} (<0.0001)
Among individuals within populations	137	438.99	−0.355 Vc	−9.04	F_{IS} (1.0)
Within individuals	145	567.5	3.91 Vd	99.72	F_{IT} (0.96)
AMOVA between tributaries within the Tippah River watershed					
Among populations	3	35.96	0.299 Va	7.84	F_{ST} (<0.0001)
Among individuals within populations	56	179.23	−0.316 Vb	−8.29	F_{IS} (0.99)
Within individuals	60	230	3.83 Vc	100.46	F_{IT} (0.93)

populations within the Tippah River watershed show, again, a significant amount of genetic variation among populations ($F_{ST} = 0.078$, $df = 3$, $p < 0.0001$), but no significant variation among individuals within populations or within individuals. In all cases, variance among individuals within populations was a non-significant negative value. Negative values can sometimes occur because estimates are calculated by subtraction of other components of variance from the overall variance observed. Non-significant negative values indicate that there is no genetic structure (Excoffier et al. 1992, ARLEQUIN website http://cmpg.unibe.ch/software/arlequin/software/2.000/doc/faq/faqlist.htm).

Contemporary and historical migration

Migration estimates indicated dispersal occurred historically among watersheds in each major drainage. Historical dispersal is indicated among the Tippah River, Cypress Creek and Big Spring Creek watersheds within the Tallahatchie R. drainage (MIGRATE, Table 5) and between all populations across watersheds in the Yocona R. drainage (Table 6). All populations within the Tippah River watershed (Table 7) also showed evidence of past connectivity.

Comparison of historical migration rates (MIGRATE) with contemporary rates (BAYESASS) revealed significantly less dispersal among watershed units within the Tallahatchie R. drainage ($t = 7.18$, $df = 5$, $p < 0.0004$) (Table 8), the Yocona R. drainage ($t = 3.63$, $df = 5$, $p < 0.008$) (Table 9), and among sites within the Tippah River watershed unit ($t = 5.49$, $df = 11$, $p < 0.0001$) (Table 10). However, in most cases 95% confidence intervals overlap between estimates, mainly due to wide confidence intervals generated by BAYESASS. Ancestry analysis (from STRUCTURE data) did not identify any migrant individuals in any population.

Table 5 Mean (±95% CI) historic migration rate (migrants per generation, N*m*) estimates from MIGRATE across watersheds in the Tallahatchie R. drainage

Migration into	Big Spring Creek (1)		Tippah River (2)		Cypress Creek (3)	
	From 2	From 3	From 1	From 3	From 1	From 2
Mean	1.86	1.34	2.28	2.16	1.25	1.32
Lower 95% CI	1.56	1.1	1.95	1.86	1.02	1.04
Upper 95% CI	2.23	1.62	2.71	2.51	1.51	1.58

Table 6 Mean (±95% CI) historic migration rate (migrants per generation, N*m*) estimates from MIGRATE across watersheds within the Yocona R. drainage

Migration into	Otoucalofa Creek (1)		Morris Creek (2)		Taylor Creek (3)	
	From 2	From 3	From 1	From 3	From 1	From 2
Mean	1.62	1.92	1.36	1.76	3.16	4.07
Lower 95% CI	1.32	1.69	1.16	1.52	2.64	3.44
Upper 95% CI	1.85	2.18	1.59	2.04	3.77	4.83

Table 7 Mean (±95% CI) historic migration rate (migrants per generation, N*m*) estimates from MIGRATE across tributaries within the Tippah River watershed

Migration into	Chili Creek (1)			Chewalla Creek (2)			Yellow Rabbit Creek (3)			Tippah R. Tributary (4)		
	From 2	From 3	From 4	From 1	From 3	From 4	From 1	From 2	From 4	From 1	From 2	From 3
Mean	0.62	0.73	0.37	1.82	3.47	3.37	2.71	2.99	2.7	1.14	1.81	1.56
Lower 95% CI	0.46	0.58	0.28	1.27	2.81	2.63	2.11	2.26	2.08	0.9	1.48	1.26
Upper 95% CI	0.77	0.9	0.47	2.34	4.33	4.21	3.41	3.74	3.42	1.44	2.21	1.92

Table 8 Mean (±95% CI) contemporary migration rate (migrants per generation, N*m*) estimates from BAYESASS across watersheds in the Tallahatchie R. drainage

Migration into	Big Spring (1)		Tippah River (2)		Cypress Creek (3)	
	From 2	From 3	From 1	From 3	From 1	From 2
Mean	0.55	0.32	0.83	0.42	0.44	0.62
Lower 95% CI	0.004	0.004	0.008	0.006	0.005	0.005
Upper 95% CI	2.7	1.65	3.64	1.87	1.94	2.83

Table 9 Mean (±95% CI) contemporary migration rate (migrants per generation, N*m*) estimates from BAYESASS across watersheds within the Yocona R. drainage

Migration into	Otoucalofa Creek (1)		Morris Creek (2)		Taylor Creek (3)	
	From 2	From 3	From 1	From 3	From 1	From 2
Mean	0.66	1.44	0.017	0.16	0.273	0.33
Lower 95% CI	0.01	0.105	0.001	0.001	0.002	0.002
Upper 95% CI	2.75	4.59	0.71	0.67	1.21	1.56

Table 10 Mean (±95% CI) contemporary migration rate (migrants per generation, N*m*) estimates from BAYESASS across tributaries within the Tippah River watershed

Migration into	Chilli Creek (1)			Chewalla Creek (2)			Yellow Rabbit Creek (3)			Tippah R. Tributary (4)		
	From 2	From 3	From 4	From 1	From 3	From 4	From 1	From 2	From 4	From 1	From 2	From 3
Mean	0.18	0.15	0.15	1.42	0.82	0.82	0.65	0.63	0.67	0.22	0.27	0.22
Lower 95% CI	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Upper 95% CI	0.99	0.84	0.84	5.72	5.11	4.97	2.45	3.26	2.9	1.03	1.46	1.13

Discussion

Our study has four primary conclusions. First, although overall patterns of population structure are similar between the two major river drainages inhabited by the Yazoo darter, the Yocona R. populations show significantly less genetic diversity and a higher degree of population structure. Second, populations within the major drainages are highly structured with almost all genetic variation partitioned among populations and not among watersheds. Third, relative to historical rates, contemporary dispersal has declined among headwater streams in both major river drainages, likely as a result of habitat loss and alteration. Fourth, roughly half of the populations analyzed possessed significant excess heterozygosity relative to Hardy–Weinberg expectations, perhaps due to small population size and binomial sampling error.

Cluster analyses

Population structure analyses (GENELAND, STRUCTURE) recovered eleven genetically distinct populations from our sampled sites (Fig. 1). This is corroborated by significant differences in F_{ST} values between populations, significant results of population differentiation tests and the results of our AMOVA analyses that identified significant genetic variation among populations. GENELAND results showed that sample sites in tributaries separated by larger, straightened, channelized stream reaches such as four of the five sites (a1, b2, c3, d4, e4) in the Tippah River watershed ($\sim$970 km^2) and the two sites (l9, m10) in the Yocona R. watershed ($\sim$763 km^2) were genetically isolated, but sample sites separated by smaller, relatively unaltered stream reaches were grouped together such as the three sites (g6, h6, i6) in the Puskus Creek watershed ($\sim$32 km^2) upstream of Puskus Lake and the four sites (n11, o11, p11, q11) in the Otoucalofa Creek watershed ($\sim$251 km^2). Although sample sites upstream (g6, h6, i6) and downstream (j7) of Puskus Lake were separated in the analyses, those upstream (d4) and downstream (e4) of Chewalla Lake (constructed in 1966, $\sim$29–43 generations) were not, possibly as a result of small sample sizes relative to samples from up- and downstream of Puskus Lake. An important point, however, is that GENELAND clustering was powerful enough to detect recently isolated populations because Puskus Lake was only impounded in 1962 ($\sim$31–47 generations).

Genetic diversity and population structure

Our finding that populations in the Yocona R. drainage have less genetic diversity and a higher degree of structuring relative to the Tallahatchie R. drainage is important. Differences in the amount of genetic diversity present in the populations endemic to each drainage can be explained by the amount of suitable habitat available to darters in the two different drainages. Currently, the Yazoo darter is known from only four small tributaries of the Yocona R. and the Otoucalofa Creek watershed within the Yocona R. drainage (Sterling et al. 2011). Past workers noted that the Yazoo darter appears to be limited to groundwater dependent perennial streams (Thompson and Muncy 1986; Suttkus et al. 1994), and this type of habitat appears to be less common in the smaller ($\sim$1,014 km^2) Yocona R. drainage than in the larger ($\sim$2,755 km^2) Tallahatchie R. drainage. This may limit the number of genetically distinct populations and effective population sizes relative to the Tallahatchie R. Our measures of allelic richness and heterozygosity from the Yocona R. drainage are similar to estimates obtained for *Etheostoma nuchale*, a darter endemic to several springs of the upper Black Warrior River drainage in northern Alabama (Fluker et al. 2010) with few populations and small effective population sizes.

Another, non-exclusive possibility is that the Yocona R. drainage has experienced more anthropogenic disturbance and fragmentation than the Tallahatchie R. drainage. Of 93 known locations with Yazoo darters, 63 are on or within 2 km of land managed by state or federal agencies. Of these 63 locations, 53 are within the Tallahatchie R. drainage and only seven are within the Yocona R. drainage (Sterling et al. 2011). Populations in the Tallahatchie R. drainage are mostly on land managed by state and federal agencies which may have afforded them some protection from disturbance over the last 70 years. In contrast, little to no protection exists for streams in the Yocona R. drainage where agriculture is more widespread, and the cities of Water Valley and Oxford continue to expand. For example, Oxford's population increased by 61% from 2000 to 2010 with densities increasing from 742 to 1,194 persons per square mile. Increased disturbance and concomitant fragmentation may have contributed to smaller effective population sizes which would increase rates of genetic drift.

Differences in the degree of population structure between the two drainages suggest that populations in the Yocona R. drainage have undergone greater genetic drift which implies that population sizes in the Yocona R. drainage are smaller than the Tallahatchie R. drainage. Evidence for this is shown in the lower measures of allelic richness and expected heterozygosity in the Yocona R. drainage compared to the Tallahatchie R. drainage and other *Etheostoma* species (Table 2). In addition, there were significantly lower levels of allelic richness, observed heterozygosity and unbiased gene diversity in the Yocona R. drainage than in the Tallahatchie R. drainage (FSTAT).

Because measures of genetic diversity are influenced by sampling design and effort, and because some confidence

intervals overlapped (e.g. H_O, Table 2), these results must be interpreted with caution. However, the potential risks of on-going loss of genetic diversity should be of concern to managers. Clearly, those populations isolated in smaller watersheds may be at greater risk relative to populations in larger watersheds within each drainage.

The finding of significant isolation by distance may reflect lingering effects of historic dispersal and genetic structure and is not a surprising result for a small, benthic, headwater fish (Johnson 2007; Beneteau et al. 2009; Lamphere and Blum 2012, however, see Turner and Trexler 1998). Partitioning of most genetic variation among populations, rather than among watersheds, is also not surprising given the significant F_{ST} scores between all populations and significant genic population differentiation. Thus, populations are experiencing low levels of migration not only across the largest, channelized stream reaches in the mainstem of the Tallahatchie R. and Yocona R. but also across the smaller, channelized stream reaches separating populations within watersheds such as the Cypress Creek watershed (e.g. sites j7, k8). If most of the variation had been partitioned among watersheds, this would indicate that the largest stream reaches were barriers, but the smaller ones within watersheds were not. Not surprisingly, we did find that when comparing the Tallahatchie R. and Yocona R. drainages, most variation was found between the two drainages. This supports the findings of Powers and Warren (2009) which estimated sequence divergence between the two drainages as 1.3% (SE $\pm$ 0.3), concluded that Yazoo darters in the two drainages were reciprocally monophyletic, and suggested that populations in the two drainages were separated by the creation of unsuitable habitat due to changing environmental and geologic conditions at the end of the Pleistocene.

Contemporary and historical migration

Analyses of historical gene flow show that individuals in the past could disperse across watersheds through the mainstem of the major river drainages and across tributaries within watersheds. Estimates of the number of effective migrants per generation (N*m*) are all above 1.0 except for migration into Chilli Creek (Tippah R. Watershed Unit) (Table 7). If this is correct, before European settlement, populations of Yazoo darters within the Yocona R. and Tallahatchie R. drainages likely were far less structured than they are today.

We detected significant differences between historic and contemporary migration rates among watershed units and even among sites within a watershed (Tippah R. unit). However, the wide 95% confidence intervals around mean estimates of contemporary migration (BAYESASS) render conclusions more uncertain than the estimates for historical migration. Even so, the overall low contemporary mean rates of dispersal are well supported by the lack of migrants detected (STRUCTURE) and by the high degree of structure found between populations, which indicate contemporary dispersal among populations is <1.0 effective migrant per generation. This agrees with all contemporary estimates of mean migration except for two (Chewalla and Otoucaolfa creeks), which we question. If rates of migration were as high as estimated in these two cases, pairwise F_{ST} scores between these populations would not be as large (0.2 and 0.09, respectively), would not be significantly different, and tests of population differentiation would not be significant. Further, as mentioned, we found a significant difference between contemporary and historical rates of migration. For these reasons, we conclude that these two estimates are anomalous, and that all populations are effectively isolated.

The weight of evidence suggests that little contemporary migration occurs among populations and across larger, channelized streams. In particular, it appears that the channelized mainstem of the Yocona, Tallahatchie, and the Tippah Rivers are barriers to dispersal. As discussed earlier, cluster analysis grouped individuals from sites in smaller and relatively undisturbed watersheds which indicate that there is a high enough rate of dispersal in those watersheds to create panmictic populations. Because there is apparent dispersal across smaller, unchannelized streams and historically high rates of dispersal across larger streams, the most parsimonious explanation for current population isolation is habitat modification and fragmentation.

Excess heterozygosity

Explanations for negative F_{IS} scores include natural selection favoring heterozygotes (Bensch et al. 2006; Reed 2007), negative assortative mating (Hallerman et al. 2003), or small populations where allele frequencies are, by chance, different between the sexes (Rasmussen 1979; Pudovkin et al. 1996). Negative F_{IS} scores are relatively uncommon in the literature and in vertebrates are most associated with polygynous social mammals such as bats or prairie dogs (Storz 1999; Storz et al. 2001). In these cases, negative F_{IS} scores result from male dispersal, female philopatry, polygyny, and active avoidance of mating among kin. In such a social structure, allele frequencies are different between females and the few males that successfully breed, resulting in elevated levels of heterozygosity.

Our data show that populations have become isolated which implies that they have become smaller, but whether or not populations have become small enough for negative F_{IS} scores due to binomial sampling error is not known. Because our data do not allow us to assign a cause for negative F_{IS} scores, investigation of demographic parameters, mode of dispersal, and mating system may help reveal the

mechanism(s) producing this interesting finding. However, because excess heterozygosity thus far is unknown in other *Etheostoma* species, it seems more likely to be a result of demographics than behavior, mating system, or selection.

Management implications

Within the Yocona R. drainage, populations appear to be genetically depauperate and to have a higher degree of population isolation relative to the Tallahatchie R. drainage. Far fewer known populations of Yazoo darters exist within the Yocona R. than the Tallahatchie R. drainage. There is also next to no protection for streams within the drainage, and anthropogenic pressure is rapidly increasing on these streams (unpubl. data; person. observ.). For these reasons, management action appears to be more imperative for the Yocona R. drainage.

Though our results demonstrate that declines in effective population sizes due to population fragmentation almost certainly occured, we do not know when these declines took place, how severe they may have been or if populations are currently stable. Further investigation of demographic parameters and modes of dispersal among streams are absolutely critical to understanding evolutionary processes and effective management of this species. Translocation of individuals among streams within each river drainage to restore genetic diversity and stream restoration aimed at restoring connectivity among populations should be carefully investigated as management options.

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References

Adams SB, Warren ML Jr, Haag WR (2004) Spatial and temporal patterns of fish assemblages of upper coastal plain streams, Mississippi, USA. Hydrobiology 528:45–61

Beerli P (2010) MIGRATE version 3.2: a maximum likelihood and Bayesian estimator of gene flow using the coalescent. http://popgen.scs.edu/migrate.html. Accessed 5 December 2010

Beerli P, Felsenstein J (1999) Maximum likelihood estimation of migration rates and population numbers of two populations using a coalescent approach. Genetics 152:763–773

Beneteau CL, Mandrak NE, Heath DD (2007) Characterization of eight polymorphic microsatellite DNA markers for the greenside darter, *Etheostoma blennioides* (Percidae). Mol Ecol Notes 7:641–643

Beneteau CL, Mandrak NE, Heath DD (2009) The effects of river barriers and range expansion of the population genetic structure and stability in greenside darter (*Etheostoma blennioides*) populations. Conserv Genet 10:477–487

Benjamini Y, Yekutieli D (2001) The control of false discovery rate under dependency. Ann Stat 29:1165–1188

Bensch S, Andren H, Hansson B, Pedersen HC, Sand H, Sejberg D, Wabakken P, Akesson M, Liberg O (2006) Selection for heterozygosity gives hope to a wild population of inbred wolves. PLoS ONE. doi:10.1371/journal.pone.0000072

Bohonak AJ (2002) IBD (isolation by distance): a program for analyses of isolation by distance. J Hered 93:153–154

Chapman SS, Griffith GE, Omernik JM, Comstock JA, Beiser MC, Johnson D (2004) Ecoregions of Mississippi, (color poster with map, descriptive text, summary tables, and photographs): Reston, Virginia, U.S. Geological Survey (map scale 1:1,000,000). http://www.epa.gov/wed/pages/ecoregions/ms_eco.htm. Accessed 20 February 2011

Chiucchi JE, Gibbs HL (2010) Similarity of contemporary and historical gene flow among highly fragmented populations of and endangered rattlesnake. Mol Ecol 19:5345–5358

Cooper CM, Knight SS (1991) Water quality cycles in two hill land streams subjected to natural, municipal, and non-point agricultural stresses in the Yazoo Basin of Mississippi, USA (1985–1987). Organ Int Ver Theor Angew Limnol 24:1654–1663

Edberg KL (2009) The effects of a reservoir on genetic isolation in two species of darters. Thesis, Western Kentucky University

Estes-Zumpf WA, Rachlow JL, Waits LP, Warheit KI (2010) Dispersal, gene flow, and population genetic structure in the pygmy rabbit (*Brachylagus idahoensis*). J Mammal 91:208–219

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611–2620

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491

Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: an integrated software package for population genetic analysis. Evol Bioinform Online 1:47–50

Falush D, Stephens M, Pritchard JK (2003) Inference of population structure: extensions to linked loci and correlated allele frequencies. Genetics 164:1567–1587

Fluker BL, Kuhajda BR, Lang NJ, Harris PM (2010) Low genetic diversity and small long-term population sizes in the spring endemic watercress darter, *Etheostoma nuchale*. Conserv Genet 11:2267–2279

Gabel JM, Dakin EE, Freeman BJ, Porter BA (2008) Isolation and identification of eight microsatellite loci in the Cherokee darter (*Etheostoma scotti*) and their variability in other members of the genera *Etheostoma, Ammocrypta,* and *Percina*. Mol Ecol Res 8:149–151

George AL, Caldieraro JB, Chartrand KM, Mayden RL (2008) Population genetics of the blue shiner, *Cyprinella caerulea*. Southeast Nat 7:637–650

Goudet J (2001) FSTAT version 2.9.3, a program to estimate and test gene diversities and fixation indices. http//:unil.ch/izea/softwares/fstat.html. Accessed 5 December 2010

Guillot G, Santos F (2009) A computer program to simulate multilocus genotype data with spatially auto-correlated allele frequencies. Mol Ecol Res 9:1112–1120

Guillot G, Estoup A, Mortier F, Cosson J-F (2005) A spatial model for landscape genetics. Genetics 170:1261–1280

Hallerman E, Brown B, Epifanio J (2003) An overview of classical and molecular genetics. In: Hallerman EM (ed) Population genetics: principles and applications for fisheries scientists. American Fisheries Society, Bethesda, pp 3–20

Haponski AE, Bollin TL, Jedlicka MA, Stepien CA (2009) Landscape genetic patterns of the rainbow darter *Etheostoma caeruleum*: a catchment analysis of mitochondrial DNA sequences and nuclear microsatellites. J Fish Biol 75:2244–2268

Hubisz M, Falush D, Stephens M, Pritchard JK (2009) Inferring weak population structure with the assistance of sample group information. Mol Ecol Res 9:1322–1332

Hudman SP, Grose MJ, Landis JB, Skalski GT, Wiley EO (2008) Twenty-three microsatellite DNA loci for population genetic studies and parentage assignment in orangethroat darter, *Etheostoma spectabile*. Mol Ecol Res 8:1483–1485

Jelks HL, Walsh SJ, Burkhead NM, Contreras-Balderas S, Diaz-Pardo E, Hendrickson DA, Lyons J, Mandrak NE, McCormick F, Nelson JS, Plantania SP, Porter BA, Renaud CB, Schmitter-Soto JJ, Taylor EB, Warren ML Jr (2008) Conservation status of imperiled North American freshwater and diadromous fishes. Fisheries 33:372–407

Jensen JL, Bohonak AJ, Kelley ST (2005) Isolation by distance, web service. BMC Genet 6:13. Version 3.16. http://ibdws.sdsu.edu/. Accessed 1 March 2011

Johnson RL (2007) Geneflow and genetic structuring of yellow cheek darters (*Etheostoma moorei*) in the little Red River watershed. Final report submitted to the Arkansas Game and Fish Commission, Little Rock, AR

Johnston CE, Haag WR (1996) Life history of the Yazoo darter (Percidae: *Etheostoma raneyi*), a species endemic to north-central Mississippi. Tulane Stud Zool Bot 30:47–60

Khudamrongsawat J, Heath LS, Heath HE, Harris PM (2007) Microsatellite DNA primers for the endangered vermilion darter, *Etheostoma chermocki*, and cross-species amplification in other darters (Percidae: *Etheostoma*). Mol Ecol Notes 7:811–813

Kuhajda BR, George AL, Williams JD (eds) (2008) The desperate dozen: southeastern freshwater fishes on the brink. Southeast Fish Counc Proc 51:10–30

Lamphere BA, Blum MJ (2012) Genetic estimates of population structure and dispersal in a benthic stream fish. Ecol Fresh Fish 21:75–86

Mantel N (1967) The detection of disease clustering and a generalized regression approach. Cancer Res 27:209–220

Narum S (2006) Beyond Bonferroni: less conservative analyses for conservation genetics. Conserv Genet 7:783–787

NatureServe (2011) http://www.natureserve.org/explorer/servlet/NatureServe?sourceTemplate=tabular_report.wmt&loadTemplate=species_RptComprehensive.wmt&selectedReport=RptComprehensive.wmt&summaryView=tabular_report.wmt&elKey=100184&paging=home&save=true&startIndex=1&nextStartIndex=1&reset=false&offPageSelectedElKey=100184&offPageSelectedElType=species&offPageYesNo=true&post_processes=&radiobutton=radiobutton&selectedIndexes=100184. Accessed 19 February 2011

Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York

Powers SL, Warren ML Jr (2009) Phylogeography of three snubnose darters (Percidae: Subgenus *Ulocentra*) endemic to the southeastern U.S. Coastal Plain. Copeia 2009:523–528

Powers SL, Mayden RL, Etnier DA (2004) Conservation genetics of the ashy darter, *Etheostoma cinereum* (Percidae: subgenus *Allohistium*), in the Cumberland and Tennessee Rivers of the southeastern United States. Copeia 2004:632–637

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Pudovkin AI, Zaykin DV, Hedgecock D (1996) On the potential for estimating the effective number of breeders from heterozygosity excess in progeny. Genetics 144:383–387

Rasmussen DI (1979) Sibling clusters and genotypic frequencies. Am Nat 113:948–951

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. J Hered 86:248–249

Reed DH (2007) Natural selection and genetic diversity. Heredity 99:1–2

Reed DH (2008) Effects of population size on population viability: from mutation to environmental catastrophes. In: Carroll SP, Fox CW (eds) Conservation biology. Oxford University Press, New York, pp 16–34

Reed DH, Teoh VH, Stratton GE, Hataway JA (2011) Levels of gene flow among populations of a wolf spider in a fragmented habitat: current versus historical rates. Conserv Genet 12:331–335

Rousset F (2008) GENEPOP '007: a complete reimplementation of the Genepop software for Windows and Linux. Mol Ecol Res 8:103–106

Shields FD, Knight SS, Cooper CM (1994) Effects of channel incision on base flow stream habitats and fishes. Environ Manag 18:43–57

Shields FD, Knight SS, Cooper CM (1998) Rehabilitation of aquatic habitats in warmwater streams damaged by channel incision in Mississippi. Hydrobiology 382:63–86

Simon A, Darby SE (1997) Disturbance, channel evolution and erosion rates: Hotophia Creek, Mississippi. In: Wang SSY, Langendoen EJ, Shields FD Jr (eds) Management of landscapes disturbed by channel incision: stabilization, rehabilitation, restoration. University of Mississippi Press, Oxford, pp 476–481

Skalski GT, Landis JB, Grose MJ, Hudman SP (2008) Genetic structure of creek chub, a headwater minnow, in an impounded river system. Trans Am Fish Soc 137:962–975

Spiegelhalter DJ (2002) Bayesian measures of model complexity and fit. J R Stat Soc Ser B (Methodol) 64:583–639

Sterling K, Warren ML Jr, Noonan BP, Reed DH, Henderson LG (2011) Yazoo darter, *Etheostoma raneyi*: population and demographic status, distributional changes, and habitat use of an endemic, nongame species. Final report submitted to the Mississippi Museum of Natural Science, Jackson MS

Storz JF (1999) Genetic consequences of mammalian social structure. J Mammal 80:553–569

Storz JF, Bhat HR, Kunz TH (2001) Genetic consequences of polygyny and social structure in an Indian fruit bat, *Cynopterus sphinx*. I. Inbreeding, outbreeding, and population subdivision. Evolution 55:1215–1223

Suttkus RD, Bailey RM, Bart HL Jr (1994) Three new species of *Etheostoma*, subgenus *Ulocentra*, from the Gulf Coastal Plain of southeastern United States. Tulane Stud Zool Bot 29:97–126

Switzer JF, Welsh SA, King TL (2008) Microsatellite DNA primers for the candy darter, *Etheostoma osburni*, and variegate darter, *Etheostoma variatum*, and cross-species amplification in other darters (Percidae). Mol Ecol Res 8:335–338

Taggart JB, Haynes RA, Prodohl PA, Ferguson A (1992) A simplified protocol for routine total DNA isolation from salmonid fishes. J Fish Biol 40:963–965

Thompson KW, Muncy RJ (1986) Darters of the Little Tallahatchie watershed in northern Mississippi. J Miss Acad Sci 31:63–77

Tonnis BD (2006) Microsatellite DNA markers for the rainbow darter, *Etheostoma caeruleum* (Percidae), and their potential utility for other darter species. Mol Ecol Notes 6:230–232

Turner TF, Robison HW (2006) Genetic diversity of the Caddo madtom, *Noturus taylori*, with comments on factors that promote genetic divergence in fishes endemic to the Ouachita highlands. Southwest Nat 51:338–345

Turner TF, Trexler JC (1998) Ecological and historical associations of gene flow in darters (Teleostei: Percidae). Evolution 52: 1781–1801

Warren ML Jr, Burr BM, Walsh SJ, Bart HL Jr, Cashner RC, Etnier DA, Freeman BJ, Kuhajda BR, Mayden RL, Robison HW, Ross ST, Starnes WC (2000) Diversity, distribution, and conservation status of the native freshwater fishes of the southern United States. Fisheries 25:7–29

Wilson GA, Rannala B (2003) Bayesian inference of recent migration rates using multilocus genotypes. Genetics 163:1177–1191