

# Consequences of seasonal drought in Ouachita mountain streams on the haplotype diversity of *Lepomis megalotis*, *Fundulus olivaceus*, and *Etheostoma whipplei*.

Love, J.W., C.M. Taylor, A.P. Rooney, and M.L. Warren, Jr. 2002

The effects of seasonal drought and habitat contraction on assemblage and the genetic structure of stream fishes have been given little attention, despite the growing literature on habitat fragmentation. During September (2001), fish assemblages were sampled from 5 isolated pool habitats in the Saline River drainage in central Arkansas during drought. Four pools occurred above a reservoir and formed during seasonal dry-down, while the fifth was part of a spring-fed system that enters the Saline river below a reservoir which separates it from the other 4 pools. Dissolved oxygen, concentration of chlorophyll a, turbidity, and variation in depth explained significant variation in fish assemblages among these pools, with pool 5 being more geographically distant and environmentally distinct than the other pools. These habitat variables will be used to investigate variation in species assemblage structure during summer drought (2002) for approximately 20 pools.

To examine genetic consequences of seasonal drought, we isolated the mitochondrial control region for *Fundulus olivaceus* and *Lepomis megalotis* collected from all 5 sites, and *Etheostoma whipplei* collected from pools 2, 4, and 5. We hypothesized, based upon available life history and immigration rate data, the degree of genetic differentiation for each species. We also hypothesized genetic differentiation was related to distance among subpopulations. Individuals from each species showed some degree of haplotype diversity, although resolution was less clear for *L. megalotis* and *F. olivaceus*. All three individuals from the three subpopulations of *E. whipplei* were genetically distinct even though the species exhibits a high immigration rate for this area. At least two haplotypes are evident for the remaining two species. Subpopulations from pool 5 were not genetically distinct from all other subpopulations for *L. megalotis* and *F. olivaceus*, suggesting that there is evidence of gene flow for these individuals. Haplotype diversity is higher for *E. whipplei* than the other species, despite their relatively high immigration rate. Although more individuals from these subpopulations must be typed, these preliminary data suggest that seasonal drought does influence the genetic structure of fishes within this system.

Fig. 1. Ouachita National Forest: Study Sites

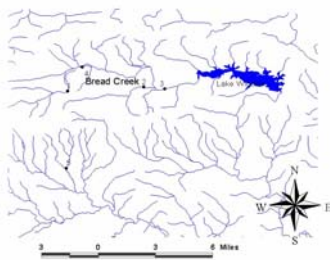


Table 3. Habitat Gradients (from Principle Components Analysis; eigenvalues > 0.60 are presented)

	PC 1	PC 2	PC 3
Elevation	-0.80		
Conductivity	0.91		
pH	0.68		
Dissolved Oxygen	0.98		
Turbidity			-0.94
Mean Depth		0.81	
cv Depth		0.98	
Chlorophyll A	-0.92		
Pool Area		-0.75	
% Variation	46%	28%	22%

## Hypotheses

- Sites will differ according to habitat variables and variation in these variables will be related to fish assemblages.
- Subpopulations of *E. whipplei*, *F. olivaceus*, and *L. megalotis* will exhibit genomic isolation by distance.
- Based upon immigration rates (Table 1) and life history strategy (Table 2), we hypothesize that *E. whipplei*, *L. megalotis*, and *F. olivaceus* will show low, high, and intermediate genetic differentiation among subpopulations.

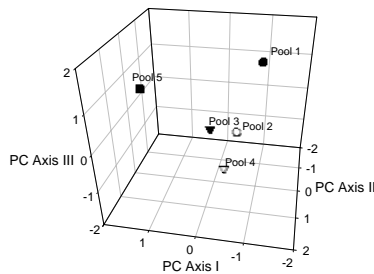
Table 1. Immigration Rates (IR) (from Taylor and Warren, 2001)

Species	IR	Avg. Abu.
<i>F. olivaceus</i>	0.241	5.3
<i>L. megalotis</i>	0.350	18.4
<i>E. whipplei</i>	1.000	9.8

Table 2. Life History Strategies (from Winemiller and Rose, 1992)

Species	Strategy
<i>F. olivaceus</i>	opportunistic
<i>L. megalotis</i>	equilibrium
<i>E. whipplei</i>	equilibrium

Fig. 2. Pool Ordination (PCA with Varimax rotation)



## Results and Discussion

- Three important habitat gradients were observed (productivity, depth, and turbidity; Table 3) and pool 5 was the most geographically distant (Fig. 1) and distinct (Fig. 2) from the remaining pools.
- F. olivaceus* and *L. megalotis* were collected in high abundance from all pools, and *E. whipplei* to a lesser extent in only pools 2, 4, and 5 (Table 4).
- Variation in fish assemblages was significantly related to habitat variables (CCA; Monte Carlo permutations,  $p < 0.05$ ; see Fig. 3).
- Mitochondrial control regions were isolated from subpopulations of the three species (Fig. 4).
- Overall, *E. whipplei* exhibited the highest haplotype diversity. *Lepomis megalotis* and *Fundulus olivaceus* exhibited less diversity and differentiation among pools (Fig. 5).
- Given these data, *E. whipplei* exhibits high subpopulation differentiation, with *L. megalotis* and *F. olivaceus* showing intermediate levels, despite IR, but consistent with life history strategies.
- Interestingly, although subpopulation 5 was geographically isolated, and distinct with regard to habitat, subpopulations of *F. olivaceus* and *L. megalotis* from pool 5 did not appear to be genetically distinct from all other forms in other subpopulations.
- Resolution with TTGE must be maximized with more individuals from each subpopulation and sequence data will be obtained to examine these relationships more closely.

Fig. 4. PCR of the mitochondrial control region run on Agarose gels. The gene is highly variable (Shedlock *et al.* 1992) and used to show polymorphism among subpopulations. Genes were isolated using published primers (Kocher *et al.* 1989; Shedlock *et al.* 1992). The forward primer for *L. megalotis* was different than that for *E. whipplei* and *F. olivaceus*.

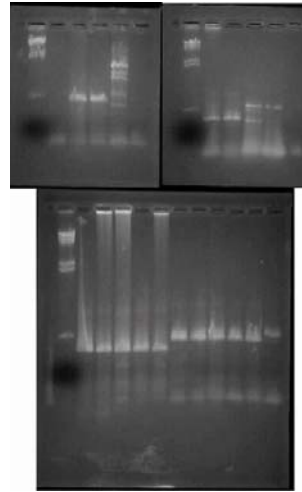


Table 4. Species Collected (in abundance)

POOLS	1	2	3	4	5
<i>Esox niger</i>	3	2	0	5	0
<i>Fundulus catenatus</i>	0	0	0	0	3
<b><i>Fundulus olivaceus</i></b>	<b>5</b>	<b>21</b>	<b>8</b>	<b>9</b>	<b>19</b>
<b><i>Lepomis megalotis</i></b>	<b>10</b>	<b>31</b>	<b>33</b>	<b>12</b>	<b>51</b>
<i>Lepomis macrochirus</i>	2	2	9	0	0
<i>Lepomis cyanellus</i>	11	7	9	9	6
<i>Micropterus punctulatus</i>	0	0	1	0	0
<i>Aphredoderus sayanus</i>	18	10	3	15	28
<i>Ictalurus natalis</i>	0	0	1	0	2
<i>Noturus lachneri</i>	1	0	0	5	4
<i>Etheostoma colletti</i>	3	2	1	12	5
<i>Etheostoma nigrum</i>	2	0	1	0	3
<b><i>Etheostoma whipplei</i></b>	<b>0</b>	<b>6</b>	<b>0</b>	<b>5</b>	<b>11</b>
<i>Percina caprodes</i>	0	0	1	0	0
<i>Camptostoma anomalum</i>	0	22	13	5	46
<i>Pimephales notatus</i>	4	0	0	12	4
<i>Erimyzon oblongus</i>	0	0	1	0	0
<i>Luxilus chrysocephalus</i>	0	0	0	0	3

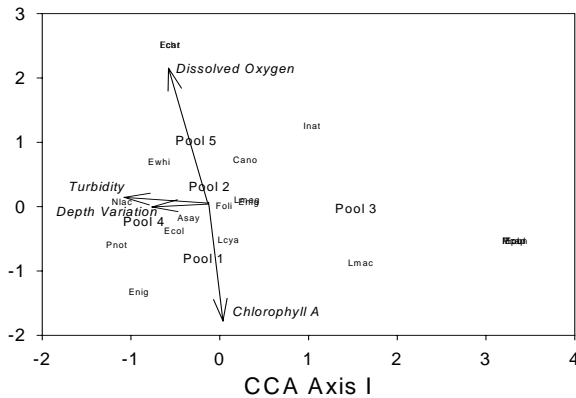


Fig. 3. Ordination of collected species and sites to dissolved oxygen, turbidity, chlorophyll a, and variation in depth. Variation of the first two axes in the canonical correspondence analysis was significantly explained by habitat gradients (Monte Carlo test;  $p < 0.05$ ).

Fig. 5. Haplotype diversity among subpopulations among sites. Variation in band number among subpopulations within a species suggests different haplotypes. Base pair differences in nucleotides were assessed using temporal temperature gradient electrophoresis (TTGE). This method creates a denaturing environment for DNA haplotypes using a constant urea density and a varying temperature (47 °C-57 °C; 1.5°C/h ramp rate).

## Acknowledgements

This project was funded with a grant from the USDA Forest Service (#SRS01CA11330127464 02010050). Travel and registration fees were provided by Mississippi State University, Department of Biological Sciences

## Literature Cited

- Kocher, T.D., W.K. Thomas, A. Meyer, S.V. Edwards, S. Paabo, F.X. Villablanca and A.C. Wilson. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proc. Nat. Acad. Sci. USA* 86: 6196-6200.
- Shedlock, A.M., J.D. Parker, D.A. Crispin, T.W. Pietsch, and G.C. Burmer. 1992. Evolution of the Salmonid Mitochondrial Control Region. *Mol. Phy. Evol.* 1: 179-192.
- Taylor, C.M. and M.L. Warren, Jr. 2001. Dynamics in species composition of stream fish assemblages: environmental variability and nested subsets. *Ecology* 82: 2320-2330.
- Smithson, E.B. and C.E. Johnston. 1999. Movement patterns of stream fishes in a Ouachita highlands stream: an examination of the restricted movement paradigm. *Trans. Amer. Fish. Soc.* 128: 847-853.
- Winemiller, K.O. and K.A. Rose. 1992. Patterns of life-history diversification in North American Fishes: implications for population regulation. *Can. J. Fish. Aquat. Sci.* 49: 2196-2217.