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Ecomorphological variation among redbreast sunfish populations of the South-Eastern United States

An Honors Thesis submitted in partial fulfillment of the requirements for Honors in Biology.

By Grayson Ariel Walker

Under the mentorship of Dr. Jamie Roberts

ABSTRACT

Individual populations of a species will morphologically adapt to their surrounding environment. It has been noted in the past that when species are placed under similar environmental conditions, they will evolve similar morphological structures and shape variation to overcome those obstacles. Redbreast sunfish (*Lepomis auritus*) were sampled from three different ecoregions (mountainous, Piedmont, and coastal plain) of 4 different isolated river basins in the southeastern North America. It was hypothesized that across basins, populations would show convergent morphological adaptations to mountain, piedmont, and coastal plain condition. I indexed using site elevation as an independent variable, serving as a proxy for ecoregion. I measured 9 morphological variables on 146 preserved redbreast specimens from 32 sites spanning all basins and ecoregions. I used a principal components analysis to visualize the variation among basins and ecoregions and generalized linear mixed models to test hypothesized relationships between each morphological variable and elevation. It was found that mountainous redbreast have smaller eyes, shorter caudal peduncles, and a rounder head shape. This may be due to the clearer waters of mountain streams and the behavior of waiting in the littoral zone as opposed to the turbulent center. However, several traits did not consistently vary with ecoregion in the hypothesized way, suggesting that basin effect cannot be ignored on redbreast morphology.

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Introduction

Genetic and morphological diversity within a species is necessary for a species to adapt to their environment. As species evolve to become better suited to their environment, they can increase their fitness and ensure long term species survival. While a whole species does grow and adapt through morphological and genetic changes, sub populations of a species are also able to adapt and evolve amongst themselves to their unique habitat. The morphological characteristics amongst a sub population are dependent upon the traits currently present in that sub population and any mutations occurring in the organisms (Kimura and Crow 1964). If populations are relatively close in proximity, or there are migratory individuals, gene flow may occur between them. Gene flow between populations can lead to a decreased genetic and morphological difference between the two, and migratory individuals could counter any effects of inbreeding by introducing new traits to a population (Gustafson et al. 2017). The ability of populations to transfer genes between the two can lead to overall species similarity and increased variation.

In contrast, when kept in isolation, a population's gene pool will consist only of its individuals as well as any mutations that may occur in successive generations. Due to this, genetic drift in two different isolated populations of the same species may result in each displaying different morphological characteristics from the other over several generations as each population evolves to meet the needs of its own habitat. For example, it has been found by J Brinsmead and M. G. Fox that external morphology of stream populations of pumpkinseed and rock bass significantly differed from lake populations (2002), as stream fishes were more slender-bodied than their lake counterparts. This brings about the question on what would happen if two populations were isolated, yet remained under similar biotic and abiotic

constraints. Would each still evolve separate morphological variations as they are separate populations, or would the similarities in the environment lead to what is known as convergent evolution? Convergent evolution occurs when different isolated species or populations evolve similar adaptations when placed under similar habitat variables. It has previously been seen that in different locations that have gone from river to reservoir, *Cyprinella venusta* has shown similar morphological adaptations to combat this change (Haas, Blum, and Heins 2010). This would lead me to believe that different populations of the same species living in similar conditions would show similar morphological variations.

To test these ideas of convergent evolution and to compare the variation between sub populations of one species, I looked at the redbreast sunfish. Redbreast (*Lepomis auritus*) are a sunfish species native to freshwater river basins of the eastern North America from Maine to Alabama. Due to the wide range of river basins and ecoregions in which it inhabits, redbreast makes an ideal species to look at to research morphological variation among sub habitats (Omernik and Griffith 2014). Specifically, I looked at their habitat range from the warm, large, turbid coastal plain waters, up through the Piedmont, and into the smaller mountains streams where streams are in contrast fast moving, smaller, and cold (Jenkins and Burkhead 1994).

Redbreast from four different river basins will be examined: Roanoke, James, Savannah, and Apalachicola-Chattahoochee-Flint (ACF). These river basins are ideal to use to look for evolutionary morphology as they are separated from each other by land and ocean water. Each basin does, however, cross through the same three habitats: mountains, piedmont, and coastal plain. It should be noted that each habitat does not consist of solely one ecoregion, but rather groups of EPA level 3 ecoregions (Omernik and Griffith 2014). While the Piedmont consists solely of the Piedmont, the coastal plain contains the Southeastern Plains, Southern Coastal

Plain, and Middle Atlantic Coastal Plains and the mountains consist of the Blue Ridge, Ridge and Valley, and North Central Appalachians. However, through the course of my thesis I will refer to the mountains, Piedmont, and coastal plain as ecoregions for simplicity. Through analyzing redbreast obtained from the same ecoregion in different isolated basins, it can be determined if those similar ecoregions are favoring the evolution of the same morphological traits.

Mountainous regions are characterized by small, faster moving, cold clear waters, with waterfalls possibly serving as isolation barriers (Jenkins and Burkhead 1994). Redbreasts often will seek out small pools in these waters as their suitable habitat, which may limit the areas of streams they choose to inhabit as they avoid the turbulent waters. Coastal plain regions are conversely categorized by slower, warmer waters, with wider areas for more open water fishes as well as large flood plains (Benke and Cushing 2005). There is often increased suspended sediment downstream, which can lead to decreased vision capabilities in the coastal plain. The Piedmont serves as an intermediate between the 2 ecoregions as habitat shifts from one to the other.

I expect there to be a predictable variation in morphological characteristics between redbreast across these three ecoregions, as the different environmental factors would lead to different preferred traits. I hypothesize that there will be an increase in eye diameter in coastal plain fishes, to compensate for poorer optical clarity in these environments due to the suspended sediment in the waters. I would also expect mountainous fishes to have a more fusiform, elongate body shape which would be evident by longer caudal peduncles, shorter and rounder bodies, and a less sloped head. Mountain waters are more turbulent, and a fusiform body shape could help overcome drag and allow a fish to maneuver through rocks and stronger currents than

what would be in the downstream coastal plain (Maia et al. 2015)(Blake 1983). I additionally hypothesize that, despite isolation, the morphological trends should remain consistent across river basins due to convergent evolution in that they are adapting to fill a similar niche, despite being in different basins. Elevation at each site will be used as a proxy for ecoregion in analysis, as elevation decreases as the river basins shift from mountainous habitats, to Piedmonts, to coastal plain. This provides us with a gradient to work with when analyzing morphological characteristics across the basins.

Methods

I made my morphological measurements on preserved redbreast specimens captured in the wild by Dr. Jamie Roberts and Mr. Garret Strickland as a part of Garret's M.S. Thesis Project at Georgia Southern University. A total of 146 redbreast were sampled from 32 different sites across South Eastern North America via rod-and-reel fishing or backpacking electrofishing (Figures 4, 5, and 6). All sites were located on either the Apalachicola-Chattahoochee-Flint (ACF), James, Roanoke, or Savannah River basins. Latitude, longitude, and elevation were recorded at each site. A total of between 3 and 7 samples were obtained from each site (Table 1). Site locations were selected based on their accessibility. 2-3 sites per ecoregion per basin were selected. The total and standard lengths of each fish were recorded in the field, and each fish was fitted with a metal tag along their jaw. Samples were originally deposited in 10% formalin and then transferred to 70% ethanol for storage. All samples were collected over the Summers of 2017 and 2018 and had been stored for between 3 months to a year before I retrieved them for measurements.

I rerecorded the total and standard lengths of the specimens, along with the mass. Using electronic calipers, 5 variables were measured to the nearest millimeter directly on the specimen: body height, body width, caudal peduncle height, caudal peduncle width, and eye diameter. Body height was measured from dorsal fin to pelvic fin. Body width was measured right behind the operculum. Caudal peduncle (CP) height was measured at the midpoint of the CP, and CP width was measured along that line in the center of the CP. Each variable was measured by the same individual with the same calipers to prevent measurement bias, and specimens were photographed and measured in a randomized order. I photographed each fish from directly above, leaving excess space between the edges of the fish and the photograph to prevent any warping due to the lens. Each photograph contained a label with the fish ID number, as well as a ruler to be used for scaling. Fish were pinned down on a dissecting tray to ensure the specimen was flat before photographing.

After the photos were checked for clarity, they were converted into a .tps file using tpsUtil and then loaded into tpsDig, following the guidelines by Shutz (2007). First, the scale was set using the ruler for reference. Seven landmarks were placed on each fish: dorsal caudal entry, end of the lateral line, ventral caudal entry, posterior anal entry, pectoral fin entry, and pelvic fin entry (Figure 1). A curve was created along the fish from the tip of the nose to the dorsal fin entry. The curve was then converted to 5 equal spaced points. This same method was used across the eye, with 3 points being used, and then from the rear dorsal fin entry to the rear anal fin entry, with 3 points being used. The files were run through tpsUtil to convert all points to landmarks, leaving each photograph with a total of 18 landmarks. I used these scaled XY coordinates for the landmarks to derive two additional morphological measurements for analysis. I recorded the straight-line distance between points 2 and 17 as a measure of caudal peduncle

length. The distance between point 10 and the straight line between points 8 and 10 was used as a representation of head roundness (Figure 1). Two additional variables were then calculated: body roundedness (body width/ body height) and CP roundedness (CP width / CP height).

Body dimensions may scale allometrically with fish growth. To correct for any influence of fish length on individual morphological variables, each variable was regressed against standard length using the non-linear model $Y = (a \cdot SL^b) + Y_0$. Y is our variable of interest, Y_0 is the intercept, and a and b are both parameters of allometric growth (Packard 2018) (Table 3). Models were fit using the “nlin” function in R3.1.3. In all further downstream analysis, the residuals were used for the variables body height, body width, CP height, CP width, CP length, eye diameter, and head roundness. Body roundedness and CP roundedness were not correlated with standard length, so the raw values for these variables were used in downstream analysis.

I used two types of statistical analyses to examine the morphological variation among the redbreast samples. First, I ran a principal component analysis (PCA) in R using the 7 residualized variables to visualize the separation among individuals in multivariate morpho space. Plots were color and shape coded to visualize the difference between each basin and ecoregion. 4 individual plots were created to show just the variation within each basin.

Second, I used generalized linear mixed models (GLMMs) to test for relationships between each morphological variable with elevation, accounting for potential fixed effects of basin and random effects of the site. 18 linear mixed models were built in R package “lme4”, in the form of $Y = \text{Elevation} + \text{Basin} + \text{Elevation} \cdot \text{Basin} + \text{Site}$. Y was the select morphological variable of interest, X was site elevation, basin was a categorical fixed effect of one of the four basins, and site was the random effect of one of the 32 sites. For each variable, I fit five alternative models, consisting of all combinations of fixed effects (elevation only, basin only,

elevation and basin, elevation*basin, and intercept only). I selected the model with the smallest AIC as the best model for that variable (Figure 3).

Results

PCA Component 1 explained 33% of the total variation in the 7 constituent variables, and body height, body width, caudal peduncle height, and caudal peduncle width loaded the strongest on this axis (Table 2). Component 2 explained an additional 17% of the variation, with caudal peduncle length and head shape loading the strongest. Eye diameter did not appear to load strongly on either axis (Table 2). The PCA was able to provide a visual representation of the variation of the fish species, with a visible distinction of the mountain fish from the piedmont and coastal plain. Viewing the PCA plots of each basin separately showed a clearer picture (Figure 2). Each basin seems to behave differently, and basin differences may be stronger than ecoregion differences, which went against my convergent evolution hypothesis. The Roanoke and James basin appear to have a cleaner distinction between the ecoregions, where as the ACF and Savannah basins do not.

Based on the best-fitting GLMMs, eye diameter, CP length, and head roundedness were the variables that were most clearly related to elevation. Regardless of basin, head roundedness increased with elevation while eye diameter and CP length decreased with elevation. There was a basin effect present for CP length and head roundedness, indicating that the mean CP length was greater and mean head roundedness was smaller in the ACF basin than in the Roanoke or James. There was a basin \times elevation interaction for body roundedness and CP roundedness. It is noted that for four of these five variables, the best fitting model contained a basin effect. This shows that in contrast to my hypothesis, the direction of the morphological variation was not

consistently related to elevation, and that the basin of origin had to be taken into account when predicting morphological variation based on elevation.

Discussion

The general trend indicated by the data is that fish in higher elevations exhibited shorter caudal peduncles and rounder heads. While I initially believed that mountainous fish would be more fusiform, and that downstream coastal plain fish would be more laterally compressed, neither body roundedness nor CP roundedness showed a consistent relationship with elevation. The Roanoke Basin was the only one to show a positive relationship between fusiform shape and elevation; however, the other basins either had a weak or negative relationship. Eye diameter was shown to be consistently smaller at higher elevations. Eye diameter may be larger in the piedmont and coastal plain region as visibility in the water decreases. This may be due to an increase of suspended sediment downstream, as well as tannins discoloring the water.

While some individual characteristics matched my hypothesis, I was inaccurate in believing that mountain fish would have more fusiform bodies, as trends are showing them as rounder with a larger head slope and shorter caudals. While it was originally thought that changing water flow along with changing elevation could be a key factor driving morphological adaptation, in that mountain fish may need to adapt a fusiform body shape to reduce drag while swimming, there may be other variables at play here. We can also view the redbreast mountain stream habitats as more of a littoral zone, with fish avoiding rapid flow and instead waiting out on the edge for prey. In contrast, the downstream habitats may function as more lake-like, with larger open water habitats. This coincides with the findings of Robinson and Wilson (1996) who, when studying pumpkinseed (a sunfish similar to redbreast), found that a more fusiform body

was more suited for open water habitat in the benthic or limnetic zone. This can be seen in our coastal plain fishes, who have longer caudals with less steeply sloped heads, which are more fusiform shaped. Coastal plain streams will contain more open-water lake-like habitats, suitable for this body form. Analyzing stream size at these sites may lend some clarity, as a larger and slower moving stream site would behave closer to these open water habitats and can explain the body shapes found.

For other body characteristics, basin must be taken into effect when discussing trends. It can be noted that the James and Roanoke basins are very similar in the linear models' trends, as well as occupying similar regions of the PCA plots. The James and Roanoke are located closer to each other than the other rivers, which may indicate that the genetic drift between the populations occurred more recently than between the other basins when ocean levels were lower. This could have resulted in similar morphological structures and adaptations. The ACF shows little to no distinction between the ecoregions and coupled with the linear models for ACF going against many of our predictions, there may be something else at play here that was not previously considered and requires a deeper analysis.

For further analysis of these samples and the possible variation among the basins and ecoregions, DNA testing is currently underway using fin clips from the samples. Relative warp analysis of the remaining tps landmarks is to be completed to gain a better understanding of the consensus redbreast and to analyze how the different basins and elevation compare in a multivariate sense. This information will help to form a more complete picture of the morphological variation among redbreast than what I was able to obtain from looking at the morphological variables. Coupling this morphological variation with any genetic variation in the

future will lead to a greater understanding of redbreast adaptations and genetics across their southeastern habitats.

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Table 1. Site and sample data

ID	BASIN	Ecoregion	Number of Fish	Latitude	Longitude
Aycocks Creek	ACF	CP	4	31.10770000	-84.76402000
Sawhatchee Creek	ACF	CP	4	31.18066000	-85.04339000
Cooleewahee Creek	ACF	CP	4	31.40497000	-84.29995000
Soque River	ACF	MT	5	34.61799000	-83.52885000
Chattahoochee	ACF	MT	4	34.68735000	-83.71039000
Chestatee Creek	ACF	MT	5	34.52794000	-83.94023000
Centralhatchee Creek	ACF	PD	5	33.31152000	-85.10495000
New River	ACF	PD	4	33.24865000	-84.93518000
Chickahominy River	JAMES	CP	4	37.57661000	-77.33373000
Falling Creek	JAMES	CP	5	37.43959000	-77.43915000
Swift Creek	JAMES	CP	5	37.31471000	-77.49495000
John's Creek	JAMES	MT	5	37.50634000	-80.10625000
Dunlap Creek	JAMES	MT	4	37.80118000	-80.04747000
Calfpasture River	JAMES	MT	4	37.96755000	-79.49653000
Hardware River	JAMES	PD	5	37.81246000	-78.45504000
Willis River	JAMES	PD	5	37.66705000	-78.16542000
Lee's Creek	ROAN	CP	7	36.49320000	-77.62953000
Roanoke River	ROAN	CP	6	36.43240000	-77.58727000
North Fork Roanoke River	ROAN	MT	5	37.18701000	-80.35273000
South Fork Roanoke River	ROAN	MT	3	37.16287000	-80.24112000
Tinker Creek	ROAN	MT	3	37.28478000	-79.91959000
Goose Creek	ROAN	PD	5	37.26648000	-79.58728000
Quankey Creek	ROAN	CP	7	36.31856000	-77.59475000
Roanoke Creek	ROAN	PD	5	36.93089000	-78.66476000
Beaverdam Creek	SAV	CP	4	32.82144000	-81.62295000
Beaverdam Creek	SAV	CP	4	32.93724000	-81.81542000
Ebenezer Creek	SAV	CP	3	32.36429000	-81.23166000
Chatooga	SAV	MT	5	34.97347000	-83.11576000
Chauga River	SAV	MT	4	34.83308000	-83.17495000
Chatooga	SAV	MT	5	34.81562000	-83.30651000
Horn Creek	SAV	PD	4	33.66411000	-82.06774000
Steven's/Turkey Creek	SAV	PD	4	33.79460000	-82.14522000

Legend

ACF	Apalachicola-Chattahoochee- Flint
ROAN	Roanoke
SAV	Savannah
CP	Coastal Plain
MT	Mountain
PD	Piedmont

Table 2. Principle component analysis variable loadings

Variable	Component Axis 1	Component Axis 2
Residual Body Height	-0.493	-0.305
Residual Body Width	-0.516	0.041
Residual Caudal Peduncle Height	-0.496	-0.173
Residual Caudal Peduncle Width	-0.417	0.02
Residual Caudal Peduncle Length	0.152	-0.674
Residual Head Roundedness	-0.206	0.592
Residual Eye Diameter	-0.065	-0.265

Table 3. Results of nonlinear model fitting of variables to standard length

	Parameter estimates (SE)		
	a	b	c
Body height	0.099 (0.043)	1.284 (0.080)	3.145 (2.299)
Body width	0.092 (0.058)	1.114 (0.114)	-0.684 (1.581)
Caudal Peduncle Height	0.205 (0.102)	0.941 (0.088)	-1.224 (1.434)
Caudal Peduncle Width	0.627 (1.022)	0.547 (0.258)	-3.536 (3.879)
Caudal Peduncle Length	0.045 (0.029)	1.268 (0.119)	3.085 (1.460)
Head Slope	0.045 (0.052)	1.053 (0.209)	-1.763 (1.123)
Eye Diameter	2.281 (2.786)	0.403 (0.173)	-5.806 (6.140)

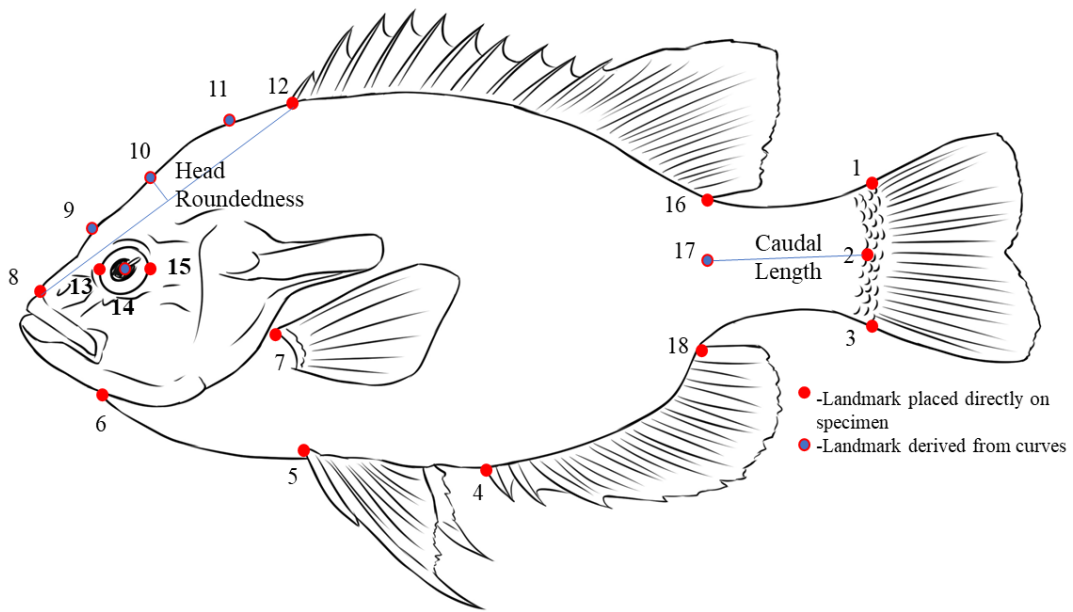


Figure 1. Diagram of redbreast with landmarks

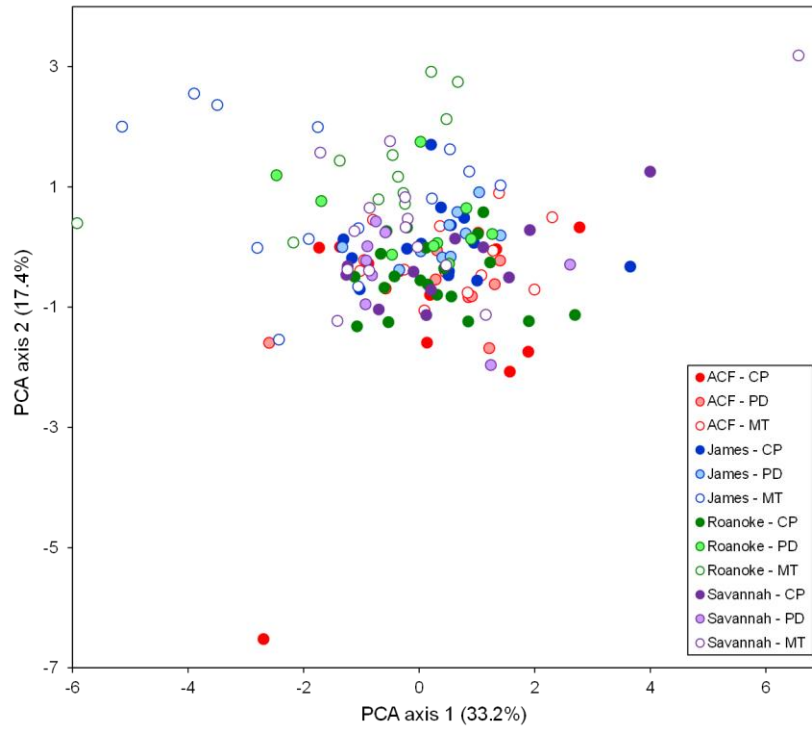


Figure 2. PCA results, as well as PCA results separated by river basin

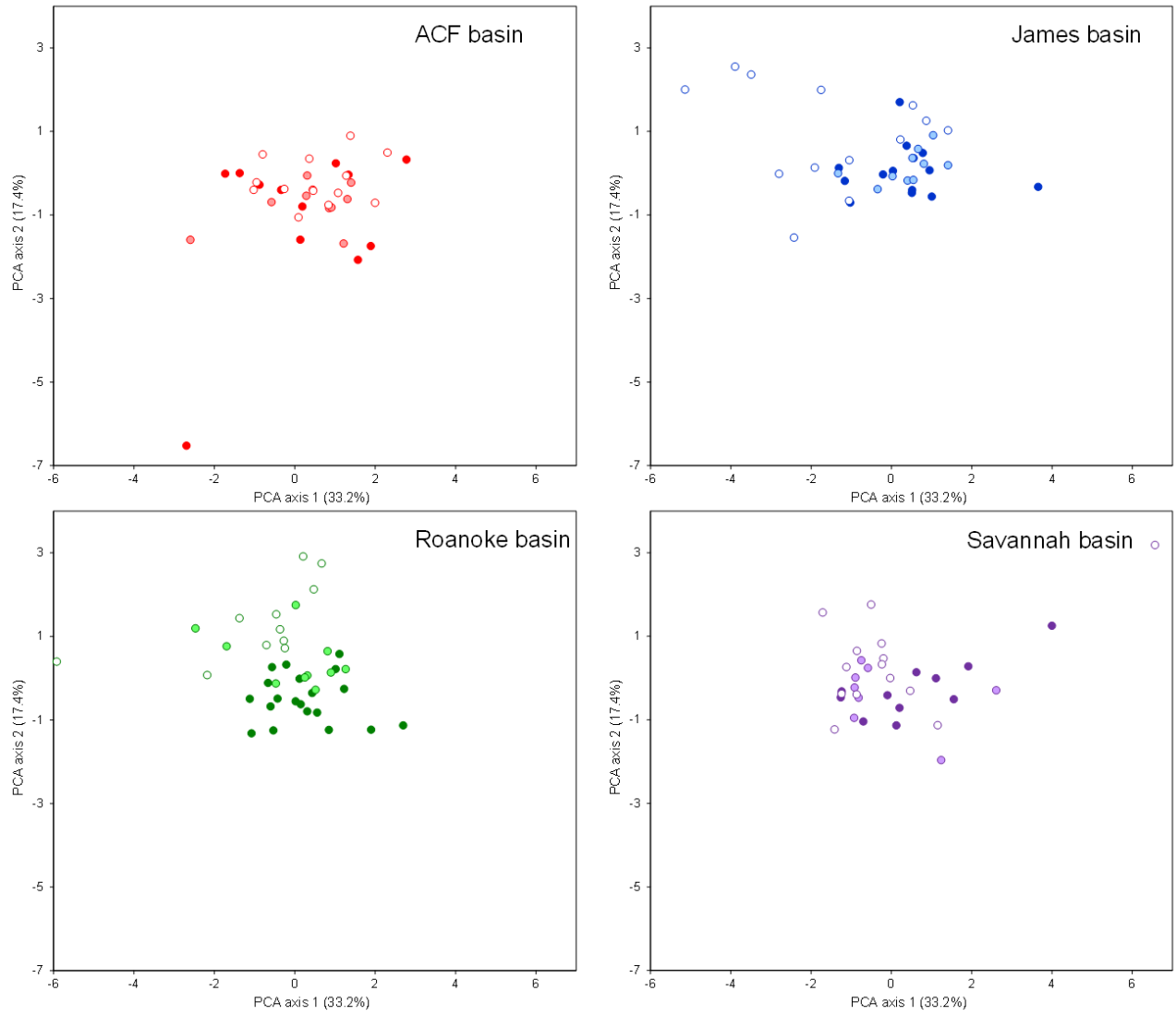
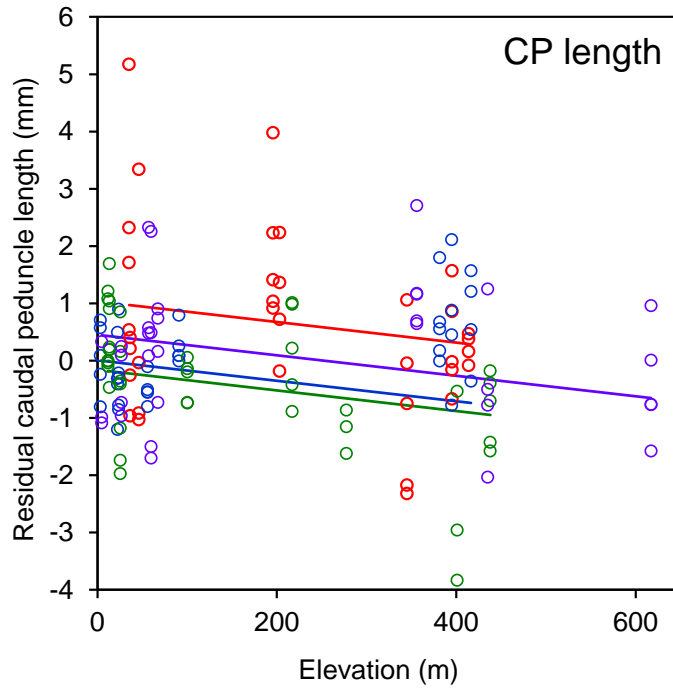
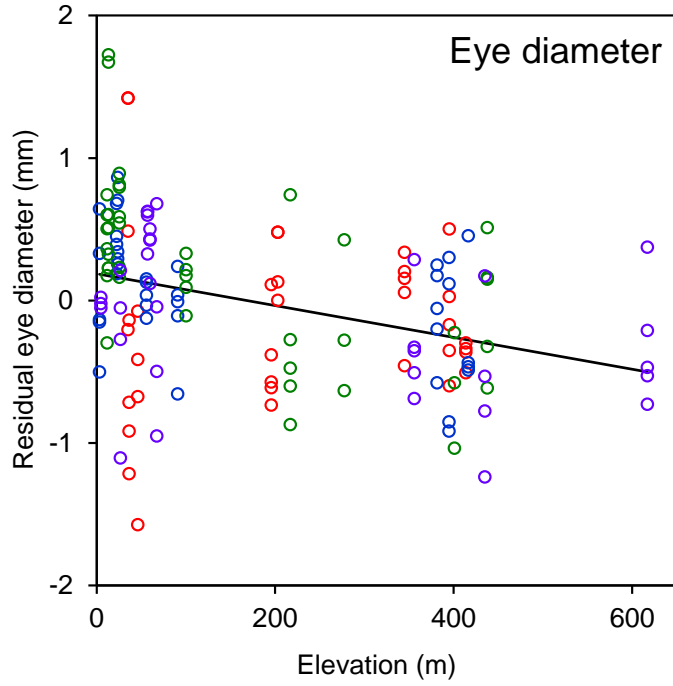
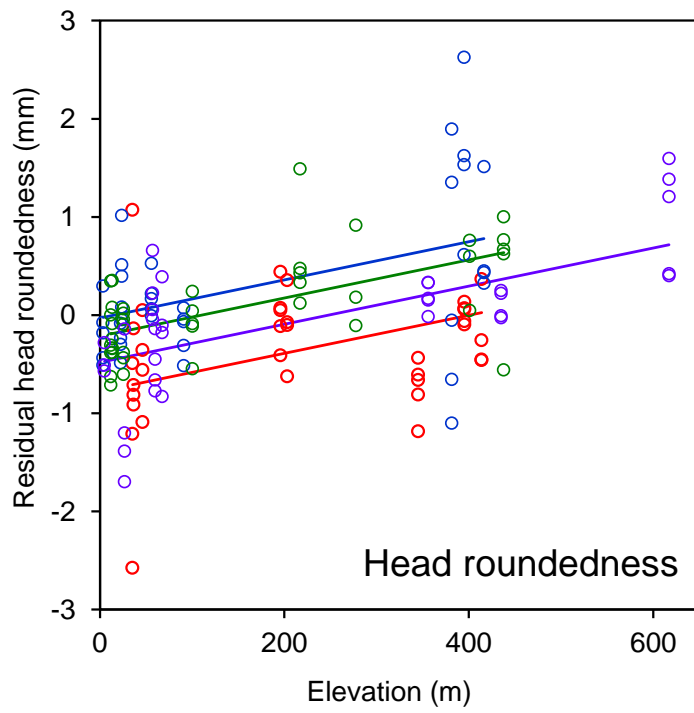
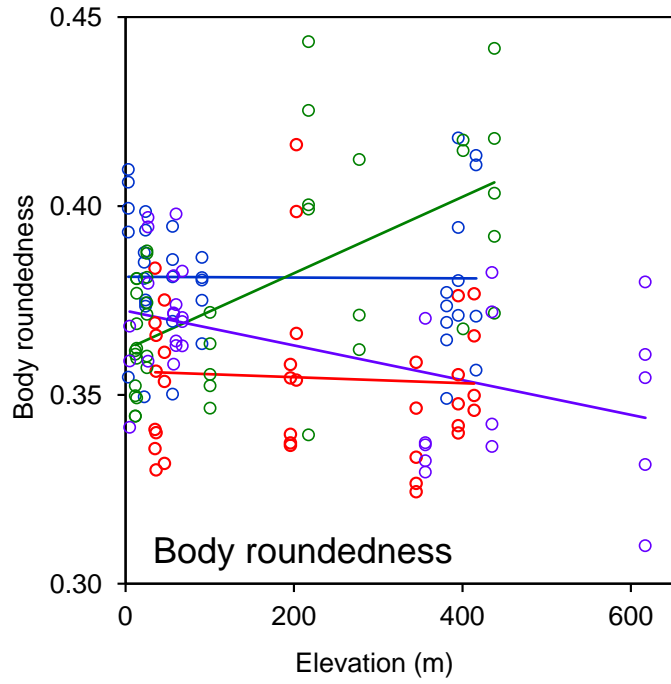


Figure 2 cont. PCA results divided by Basin





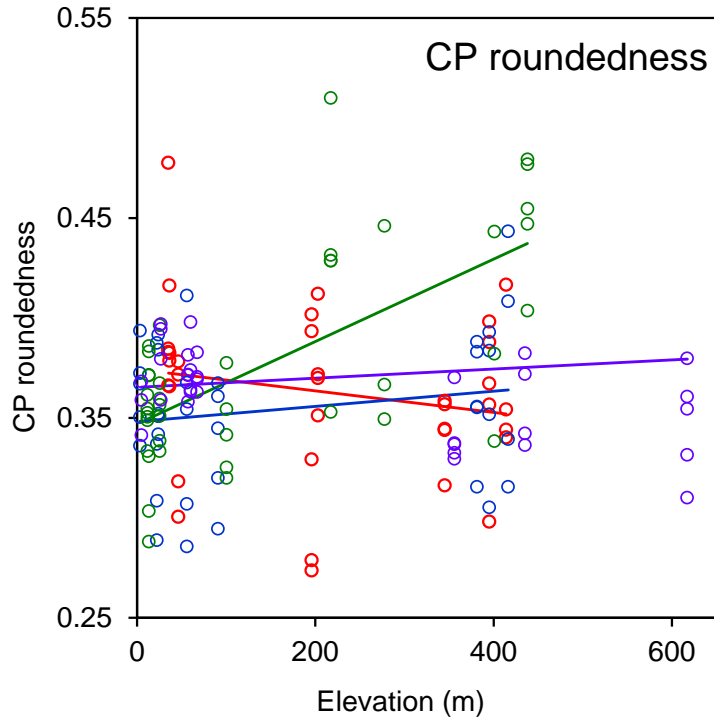


Figure 3. Scatterplots from the best generalized linear mixed models. Color Key is the same as figure 2. The best models for each were: eye diameter-elevation only, CP length- elevation + basin, head roundedness – elevation + basin, body roundedness – elevation + basin + interaction, and CP roundedness – elevation + basin + interaction.

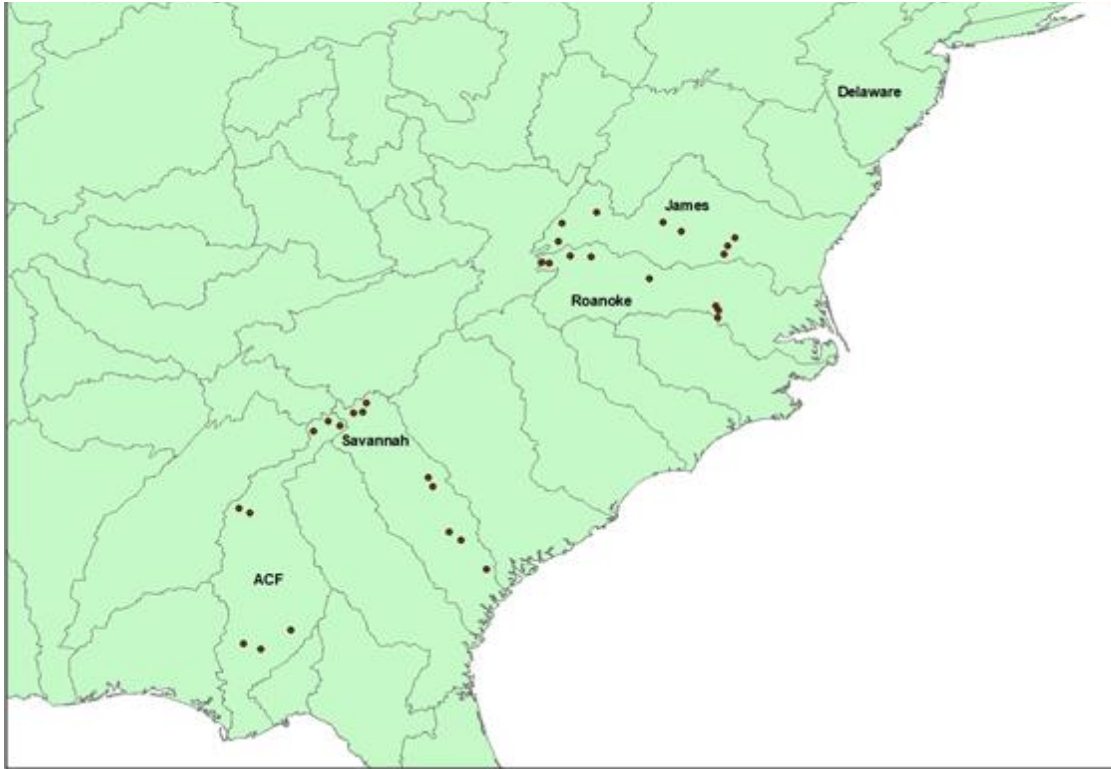


Figure 4. Map outlining the sample sites along the four river basins

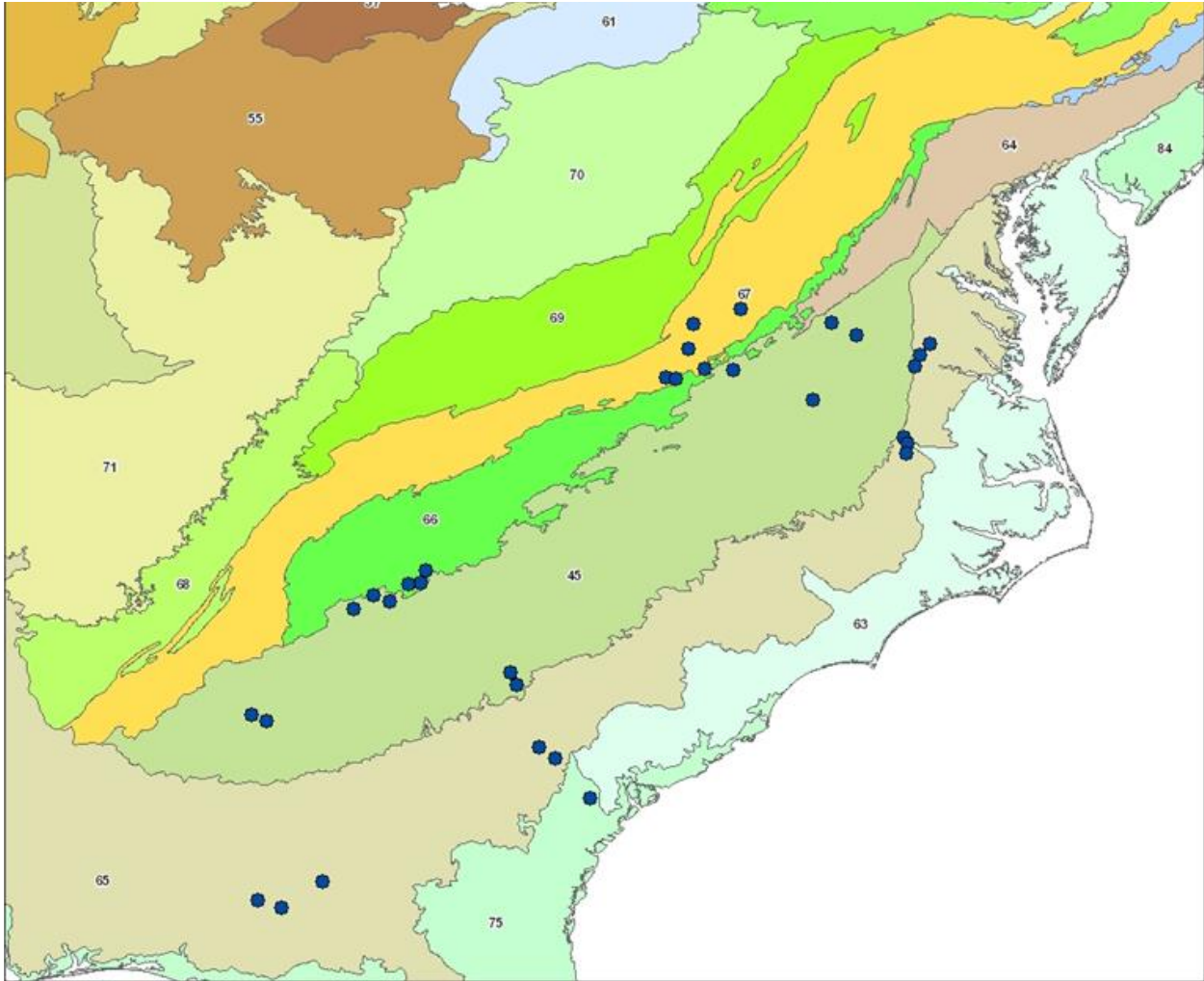


Figure 5. Ecoregion map with points indicating sampled sites. Key: 45- Piedmont, 62- North Central Appalachians 63- Middle Atlantic Coastal Plain, 65- Southeastern Plains, 66- Blue Ridge, 67- Ridge and Valley, 75- Southern Coastal Plain (Omernik and Griffith 2014). Southeastern Plain, Southern Coastal Plain, and Middle Atlantic Coastal Plain were grouped together as “coastal plain” and Blue Ridge, Ridge and Valley, and North Central Appalachians were grouped as “mountains.”

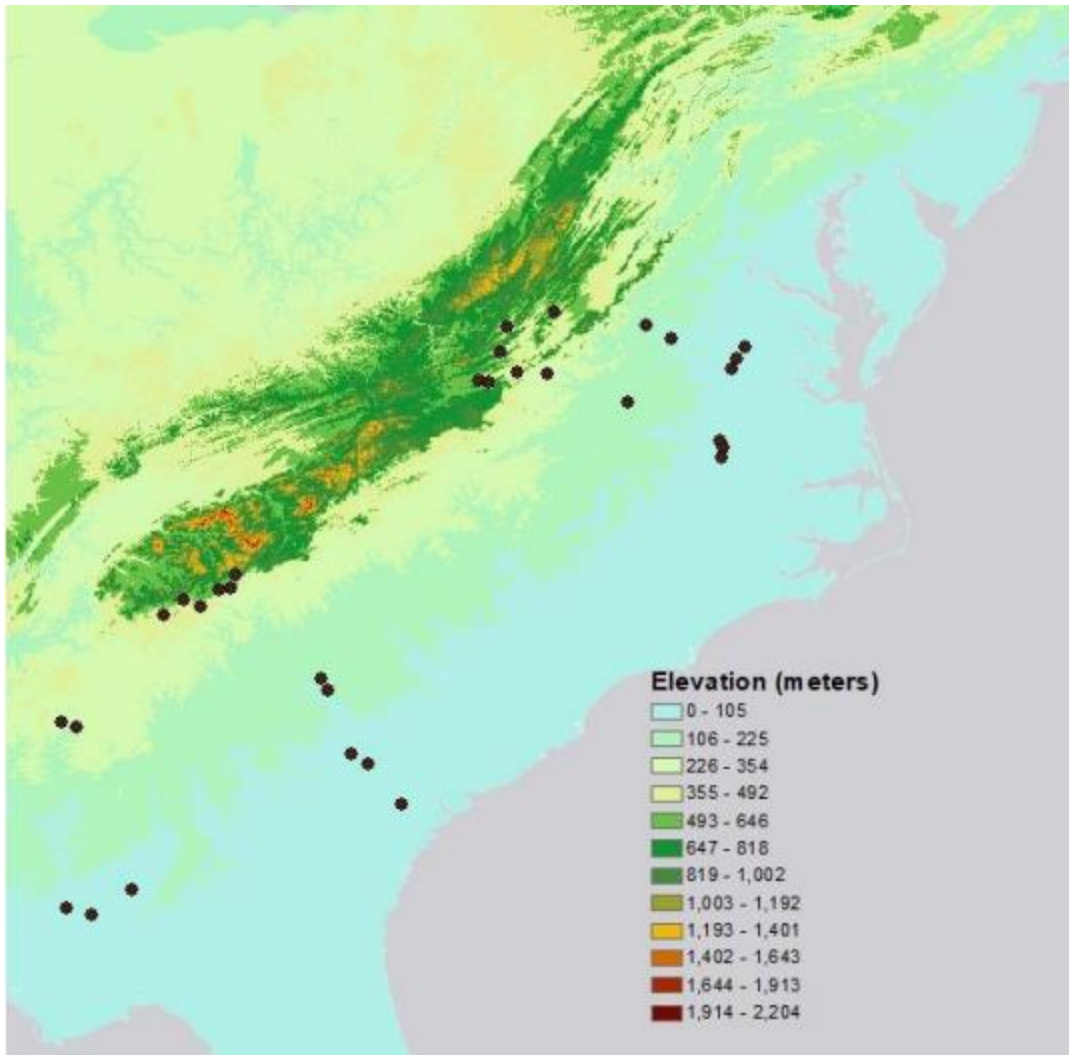


Figure 6. Elevation map of study area with sites.