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Amber Nations

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Testing for Character Displacement between Two Abundant Stream Fishes

by

Amber Nations

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Approved by:

Jacob F. Schaefer, Ph.D., Thesis Advisor,
School of Biological, Environmental and Earth Sciences

Jacob F. Schaefer, Ph.D., Director,
School of Biological, Environmental and Earth Sciences

Sabine Heinhorst, Ph.D., Dean
Honors College

ABSTRACT

Character displacement is a pattern that can be used to explain differences between similar species in sympatric and allopatric situations. Gause's Principle explains that a niche can only be occupied by one species at a time, so character displacement may be a way for similar species in the same habitat to shift resource use and compensate in order for the species to coexist. The Southeastern United States offers a unique opportunity to study this pattern because the diversity of freshwater fauna is quite high. However, the question of "How did this region become so diverse?" remains unanswered. One way for speciation to have increased diversity would involve changing an organism's biology, specifically its body morphology through character displacement. The study of how an organism's body is related to environmental factors is ecomorphology. In order to test if character displacement could have contributed to the high diversity in the Southeast, two similar species from one of the most diverse groups in this region (darters) were used, *Percina sciera* and *Percina nigrofasciata*. Geometric morphometrics was used to measure and analyze differences in body shape between individuals in sympatric and allopatric drainage systems. The largest difference seen was between the two species and while it was not considered as significant as the other results, there was a difference seen in the interaction between treatment (sympatric vs. allopatric) and species. The expanded caudal peduncle seen in the shift from *P. sciera* to *P. nigrofasciata* could be explained by the fish expanding their niche to include different habitats, such as faster flowing habitats. Future studies should look at individual drainages instead of major drainage systems to look for smaller scale changes between sympatric and allopatric individuals.

Keywords: ***character displacement, geometric morphometric, Percina, morphology***

DEDICATION

To my parents who always encourage me and my brother who could not be here to see this finished, but I think would have enjoyed the programs used.

ACKNOWLEDGMENTS

I would like to thank Dr. Schaefer for his continual grace and guidance throughout this entire process. I know that I have learned much and will carry this new knowledge with me as I continue to pursue new opportunities. I would also like to thank Loren Stearman for his help when I had questions or needed more resources and because this project would not have existed without him.

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GM	Geometric morphometric
MANOVA	Multivariate analysis of variance
PCA	Principal component analysis

CHAPTER I: INTRODUCTION

Character displacement has become a point of interest in the past few decades since the term's introduction in the 1950s. The term was first used by Brown and Wilson in 1956 to describe a pattern that could be seen when two closely related species who had similar ecological roles were found together and separately (Brown &Wilson, 1956). When the species were found in sympatry (together), they would often diverge to become slightly more different. When the species were found in allopatry (separately), they would be more similar, making it hard to distinguish the two from each other. One explanation for this pattern is Gause's Principle (1934), which states that two species cannot occupy the same niche simultaneously. High similarity in niche use is theorized to lead to one of the species outcompeting the other or a divergence between the two species, causing them to change so they no longer fill the same niche (character displacement). An organism's niche refers to its role in the surrounding community and how it interacts with the biotic and abiotic factors in that community. When species that try to fill the same niche co-occur, this will lead to competitive exclusion, causing one of the species to be extirpated or eliminated from that environment. Because the two species will fight over limited resources, one species will outcompete the other.

When competition occurs due to two species trying to occupy the same niche in the same environment, it can reduce the species' fitness. Natural selection will select for traits that limit competition and therefore maximize fitness and chances of passing their genes on to the next generation. So, if individuals within a population differ in a heritable way that allows them to occupy a different niche, if only slightly, then these individuals are less likely to experience competition. These individuals within that population now have a better chance of passing on their genes since they have access to more or different resources, which can increase their fitness. Thus, the process of character displacement should result in differences that allow similar species to coexist through the occupation of different niche spaces. Stuart et al. (2017) details how over time character displacement has come to mean a process of divergence that was due to natural selection through species interactions. In other words, character displacement is one of the patterns seen that can lead to diversification, which in turn can result in speciation. By understanding what causes speciation, we will be better able to understand how species arise and determining relationships between and among species. One of the fields of study that aims to do this is ecomorphology (Ricklefs & Miles, 1994), which involves inferring the ecology of a species, or its niche space, from that species' morphology.

Literature Review

The southeastern United States is a biodiversity hotspot when it comes to flora and fauna, especially freshwater fishes. One of the most diverse groups found here are the darters, a group within the order Perciformes, family Percidae. They are only outnumbered by minnows (family Cyprinidae) in terms of diversity (Sheldon, 1988). Darters compose about 20% of the diversity for all North American freshwater fish species (Carlson & Wainwright, 2010). This approximate 20% is composed of over 180 species in five main genera, but the majority are in the genera *Percina* and *Etheostoma* (Helfman et al., 2009). Although the genus Percina is monophyletic (meaning all members of this genus can be traced back to the same ancestral species), the species *Percina nigrofasciata* is not single taxon, but is composed of an eastern and western clade with each group having their own distinct distributions (Hayes & Piller, 2018). Most darter species are found in benthic riffle habitat (Helfman et al., 2009). They will also display sexual dimorphism, with breeding males often having bright colors or patterns in order to attract females (Helfman et al., 2009). During the spawning season, egg size is usually larger at the beginning of the season and decreases over time, but clutch size will be smaller at the beginning of the season and then grow as time goes on during the spawning season, as seen in one species of darter, *Etheostoma lynceum* (Heins et al., 2004).

In addition to the southeastern United States being a biodiversity hotspot, it is also a place of high endemism (Noss et al., 2015). Sheldon (1988) found that most species of stream fishes have limited ranges. Considering both of these, it should not seem surprising many species of darter display clumped distributions and endemism (Fluker et al., 2010, 2014; Hollingsworth & Near, 2009; Page, 1983). Because of the high levels of endemism, darter species serve as good model systems to study the mechanisms associated with evolution. These high levels of biodiversity and endemism also raise the question of what led to or caused this speciation seen in darters in this region. One possibility is that character displacement may have played a role in the diversification of these species and served as a mechanism driving their evolution. Due to aquatic species being confined to waterways, they would only be able to spread and expand their range by moving up or down streams and rivers, traveling through coastal areas, or being physically relocated, and they are not able to spread as easily as some of their terrestrial counterparts. This constraint can be compounded by artificial changes to the waterway, such as dams, as these can impede movement and separate populations or make it much more difficult/impossible for species to travel up or down to spawn. This can cause a loss in diversity or can cause the populations to diverge (Franssen, 2012; Smith et al., 2019; Valenzuela-Aguayo et al., 2020). Because of this constraint, similar species may often be in the same area within a water system or drainage (sympatric situations). These sympatric situations could have pushed the species to modify their biology in order to maximize their niche. If these modifications were significant enough, then a new species could have arisen.

One example of modifying an organism's biology would include changing its body shape or morphology. An organism's body morphology can be indicative of its ecology. For instance, thick fur or layers of blubber is indicative that a species lives in a colder environment. Species living in deserts will likely have adaptations that allow it to better survive, such as large ears to allow the organism to better cool off or having thick skin to prevent desiccation. More specifically, smaller or subtle changes can allow an organism to be better adapted to its environment or maximize fitness. The field of ecomorphology allows for these changes to be studied. Ricklefs and Miles (1994) described it as the idea that the morphology of an organism could point to what that organism's role was in its environment, its niche. So, by examining the body shape differences in a species, it may indicate that the species has changed the niche that it occupies. Ventura et al. (2017) demonstrated that the preferred prey of four *Diplodus* species (*D. sargus, D. puntazzo, D. vulgaris* and *D. annularis*) influenced their growth patterns as well as their body shape. The different prey preferences resulted in the different species filling different niches even though they were all located within the same geographical area. Nakano et al. (2020) also demonstrated that two species of char had divergent mouth and feeding morphology while in sympatry that allowed them to utilize different prey, and to reduce competitive pressure.

Near and Benard (2004) hypothesized sympatric speciation occurred faster than allopatric speciation; however, closely related species rarely diversify in sympatry which raised their question to study darters and their diversification rates (Near et al., 2000; Near, 2002; Page et al., 2003; Wiley & Mayden, 1985). The results of this study revealed that speciation occurring in logperch, 10 species in the genus *Percina*, could be attributed to allopatric processes since the highest levels of sympatry was only seen in distantly related species, resulting in rapid rates of speciation (Near & Benard, 2004). There has also been evidence of a positive correlation between co-occurring species and morphological characteristics changing, meaning that when closely related species co-occur, then certain morphological traits of the species will more rapidly change (Carlson et al., 2009). Because the results from these studies can seem contradictory, this is one of the reasons why more research is needed to understand the role of character displacement in speciation.

Geometric morphometrics (GM) is a statistical analysis that allows someone to analyze biological shape data (Mitteroecker & Gunz, 2009). The first step involves digitizing landmarks on specimens. This is followed by a Procrustes procedure to rotate and scale the landmarks to control for size, picture angle, etc. This process results in shape variables that summarize the shape variability among individuals. These shape variables are then analyzed by MANOVA or principal components analysis (PCA) or used to make deformation grids summarizing shape differences. GM allows for many types of analysis to be performed at once while also allowing the investigator to see the measurements in a space created by the map of the morphologies. In this way, outlines of the shapes and surfaces can be viewed. These generated maps can then be compared to each other to determine similarities or differences. For this project, GM would be used as a technique to detect subtle differences in shape. These differences in shape are then assumed to be linked to differences in ecology (ecomorphology) and could be examples of character displacement.

Two darter species found in sympatric and allopatric situations throughout the Southeastern United States are *Percina nigrofasciata* and *Percina sciera*. I hypothesize that there will be significant differences between the species depending on their situation (sympatric vs. allopatric). If character displacement is driving shape differences, sympatric populations will be more different in shape than allopatric populations. This will result in a strong statistical interaction between species and sympatric-allopatric classification of drainages.

CHAPTER II: METHODOLOGY

All of the specimens used in this study had been previously gathered and are a subset of USM's Ichthyology collection that are stored at Lake Thoreau Environmental Center (Table 1). Figure 1 gives the locations of the sample sites for all the specimens. *P. nigrofasciata* had been gathered from the Choctawhatchee, Pearl, Pensacola, Pascagoula, Lake Pontchartrain, Coastal Rivers drainage systems. *P. sciera* had been gathered from the Red River, Lower Mississippi, Pearl, Lake Pontchartrain, Pascagoula drainage systems. This means that all individuals from the Pearl, Pascagoula, and Lake Pontchartrain drainage systems are in sympatry while individuals from the Choctawhatchee, Pensacola, Coastal Rivers, Red River, and Lower Mississippi drainage systems are in allopatry (Table 2).

Table 1: Table detailing where the specimens were caught and which treatment they fall under (sympatric or allopatric). The table is organized by species first (first column) with all the locations that species was collected at. Each specimen was associated with a certain museum number (the second column) and field number (the third column). The treatment of the individuals from that site can be found in the fourth column. The specific body of water and drainage system the specimens came from are also given (columns five and six).

Species	**USM #**	**Field #**	**Treatment**	**Drainage system**	**Water Body**
P. nigrofasciata	52896	ICH-16-17	allopatric	Choctawhatchee	Pea River
	25340	R00-008	sympatric	Pearl	Hurricane Creek
	30121	P04-02	sympatric	Pearl	Upper Little Creek
	19659	R96-039	sympatric	Pearl	Upper Little Creek
	8403	DCH 625	allopatric	Pensacola	Alligator Creek
	20961	R97-017	sympatric	Pearl	Graves Creek
	23906	R99-031	sympatric	Pearl	Lower Little Creek

Table 1 (continued)

	48981	FS15-140	sympatric	Pascagoula	Tishkill Creek
	30485	JPS05-12	sympatric	Pascagoula	Shelton Creek
	30227	PER-001	sympatric	Pascagoula	Bowie Creek
	8359	B85-35	sympatric	Lake Pontchartrain	East Fork Amite River
	54584	CP17-2	sympatric	Pascagoula	Martin Branch
	32053	JPS 05-50	sympatric	Pascagoula	Hayden Creek
	56143	ICH 18-01	sympatric	Pascagoula	Martin Branch
	32352	BOF 06-01	sympatric	Pascagoula	Black Creek
	30248	PER-002	sympatric	Lake Pontchartrain	East Amite
	9701	R79-119	sympatric	Lake Pontchartrain	Amite River
	36297	FUN 10-21	sympatric	Lake Pontchartrain	Wagoner Creek
	19932	WTS 96-018	sympatric	Lake Pontchartrain	West Fork Amite
	19934	WTS 96-019	sympatric	Lake Pontchartrain	East Fork Amite
	21835	R98-025	allopatric	Coastal Rivers	Wolf River
	34391	FS08-79	allopatric	Coastal Rivers	Biloxi River
	51068	FS16-367	allopatric	Coastal Rivers	Flat Branch
	50992	FS16-285	allopatric	Coastal Rivers	Bayou Billie
	19251	BA96-044	allopatric	Coastal Rivers	Wolf River
P. sciera	42640	ICH-12-15	allopatric	Red River	Little River
	20545	99999 wolf	allopatric	Lower Mississippi	Indian Creek
	23905	R99-031	sympatric	Pearl	Lower Little Creek
	26653	P01-01	sympatric	Pearl	Strong River
	56166	ICH-18-02	sympatric	Pearl	Strong River

Table 1 (continued)

	26670	P01-04	sympatric	Pearl	Strong River
	10781	R91-008	allopatric	Lower Mississippi	Hatchie River
	5626	P88-059	allopatric	Lower Mississippi	Bayou Pierre
	5584	P88-053	allopatric	Lower Mississippi	Bayou Pierre
	50942	FS16-169	allopatric	Lower Mississippi	McGehee Creek
	5509	P88-045	allopatric	Lower Mississippi	Bayou Pierre
	8377	B85-29	allopatric	Lower Mississippi	Clarks Creek
	36288	FUN 10-20	sympatric	Lake Pontchartrain	West Fork of Amite
	9700	R79-119	sympatric	Lake Pontchartrain	Amite River
	19933	WTS 96-018	sympatric	Lake Pontchartrain	West Fork Amite
	36306	FUN 10-21	sympatric	Lake Pontchartrain	Wagoner Creek
	32483	JPS 06-21	sympatric	Pascagoula	Bowie Creek
	24232	MF 000-001	sympatric	Pascagoula	Bouie River
	53353	PD16-41	sympatric	Pascagoula	Okatoma Creek
	32059	JPS 05-50	sympatric	Pascagoula	Hayden Creek
	30232	PER-001	sympatric	Pascagoula	Bowie Creek

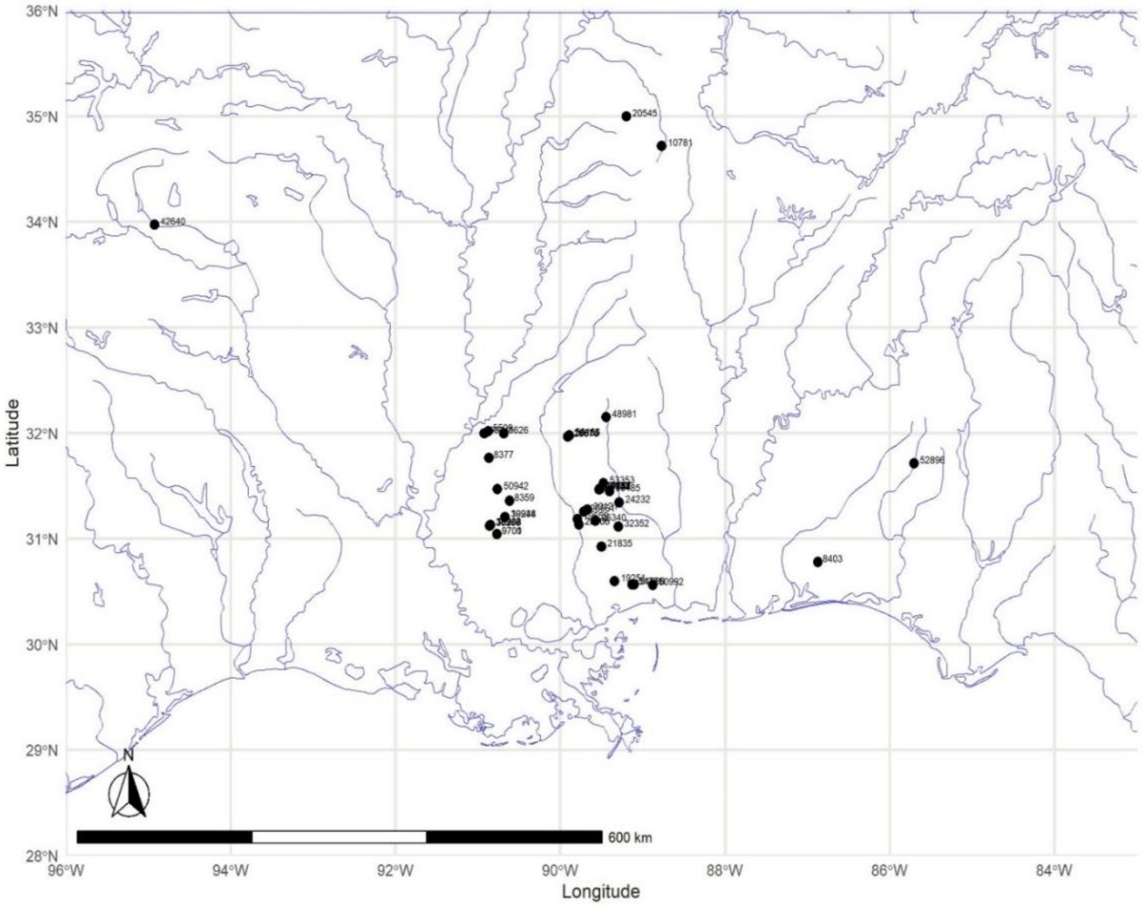

Figure 1: Map of the sample sites. Each point represents a site where samples were gathered. The number associated with each point corresponds to the USM museum numbers that can be found in Table 1 (pictured above map).

Table 2: The number of specimens per drainage system. There were eight different drainage systems that specimens came from, three of which were sympatric (Lake Pontchartrain, Pascagoula, and Pearl). The situation of the drainage system was also included. The totals of each situation (sympatric or allopatric) for each species were included to show a total of 384 specimens.

Species	**Drainage System**	**Number of Specimens**	**Sym./Allo.**
P. nigrofasciata	Choctawhatchee	5	Allopatric
	Coastal Rivers	40	Allopatric
	Lake Pontchartrain	49	Sympatric
	Pascagoula	65	Sympatric
	Pearl	47	Sympatric
	Pensacola	5	Allopatric
Total Allopatric		**50**	
Total Sympatric		**161**	

Table 2 (continued)

P. sciera	Lake Pontchartrain	25	Sympatric
	Lower Mississippi	72	Allopatric
	Pascagoula	38	Sympatric
	Pearl	31	Sympatric
	Red River	7	Allopatric
Total Allopatric		**79**	
Total Sympatric		**94**	
Total Specimens		**384**	

In preparation for the GM analysis, pictures of the left lateral side of all the specimens were taken using an LG G5 phone without the flash. In order to have a solid background and have the samples suspended, a mount was formed using wood, black felt and pins. The black felt overlays the wood bench to create a solid background for the pictures. Three pins were used in order to mount the fish in front of the background. In order to have a standard of comparison, a ruler was placed at the bottom of the field of view for all the pictures. Labels detailing each sample and individual fish were also created and added to each picture.

The pictures were then transferred to a computer to upload them into R (R Core Team, 2020). Then, using the geomorph package (Adams et al., 2020) in R (R Core Team, 2020), a total of 19 landmarks were selected on each specimen (Figure 2) based on landmarks from previous studies (Bower & Piller, 2015; Guill et al., 2003.). Once all the pictures had been digitized, the stereomorph package (Olsen & Westmeat, 2015) in R (R Core Team, 2020) was used to adjust any landmarks that needed it.

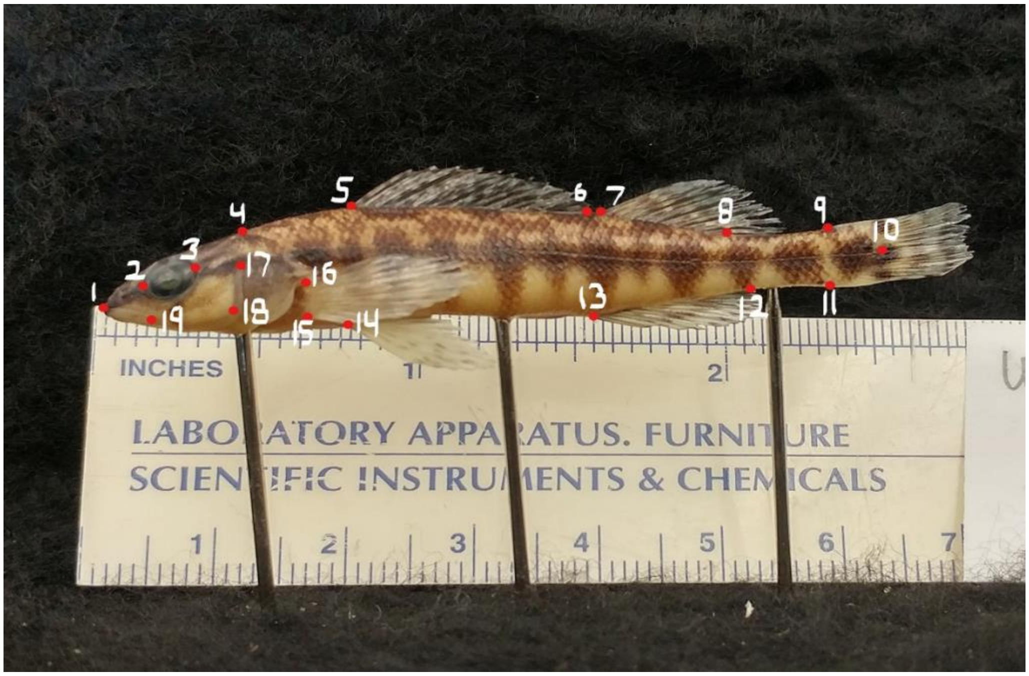

*Figure 2: Nineteen landmarks selected for GM analysis***.** 1)Tip of snout. 2)Anterior corner of eye. 3)Posterior corner of eye. 4)Nape of skull. 5)Origin of first dorsal. 6)Insertion of first dorsal fin. 7)Origin of soft dorsal. 8)Insertion of soft dorsal. 9)Top of caudal fin base. 10)Caudal peduncle. 11)Bottom of caudal fin base. 12)Insertion of anal fin. 13)Origin of anal fin. 14)Insertion of pelvic fin. 15)Bottom of pectoral fin base. 16)Top of pectoral fin base. 17)Dorsal point of cheek. 18)Angle of the cheek. 19)Angle of the mouth

To analyze the data, a Procrustes rotation and scaling was used to configure the landmarks into x and y coordinates to produce shape variables (Mitteroecker and Gunz 2009). This was then followed by Multivariate Analysis of Variance (MANOVA) and Principal Component Analysis (PCA) of the shape variable (Mitteroecker and Gunz 2009). MANOVA was used to test for differences in the shape variables by body size (allometric effects), species, treatment (allopatric or sympatric), and the interaction between species and treatment. It was predicted that the differences between treatment will be significant because this would reflect that the body morphologies change between when the species are found in sympatry vs. allopatry. PCA was used to create a scatter plot of all the x and y coordinates (Figure 3) which allowed for the data to be summarized and visualized in two dimensions.

CHAPTER III: RESULTS

The results of MANOVA (Table 3) all showed significant at $p < 0.05$ between the shape variables by body size (allometric effects), species, treatment (allopatric or sympatric), as well as the interaction between species and treatment, meaning that the body shape changed a significant amount across these different factors. The largest difference seen was between the two species (5.2% of variation, largest Z). The next biggest difference is that among the sizes (4.4% of variation, second largest Z). The treatment (sympatric and allopatric differences) was the next largest difference seen after body size (about 2% of variation). The interaction between treatment and species (testing if the two species changed differently in sympatry vs. allopatry) showed to be significant, but the Rsq and Z values were quite small (less than 1% of variation, smallest Z, 2.45) compared to the other variables.

Table 3: Results of MANOVA test. All values were found to be significant, but Csize and Species had the largest Rsq and Z values while Treatment:species had the smallest values. Z is the value from calculating the effect size. R^2 refers to the percent of variance explained.

	Df	R^2	F	Z	Significance
Csize	1	0.04383	17.5433	7.5086	0.001
Treatment	1	0.02020	8.0854	5.0412	0.001
Species	1	0.05230	20.9325	6.6473	0.001
Treatment:species	1	0.00663	2.6528	2.4578	0.012
Residuals	351				
Total	355				

The results of the PCA of shape variables (Figure 3) shows that overall, the species appear different. This is demonstrated by the separation of the mean body morphologies (Figure 3). The plot also demonstrated that the species changed similarly when in sympatry by moving up and to the right in the PCA space. The variation in the morphologies between species (Figure 4) was mostly characterized by deeper heads, longer snouts, and the shortening of the caudal peduncle. The variation in the morphologies between allopatric and sympatric (Figure 4) was mostly characterized by the expansion of the caudal peduncle. This is demonstrated by the difference in direction between landmarks 8 and 12 and landmarks 9, 10, and 11.

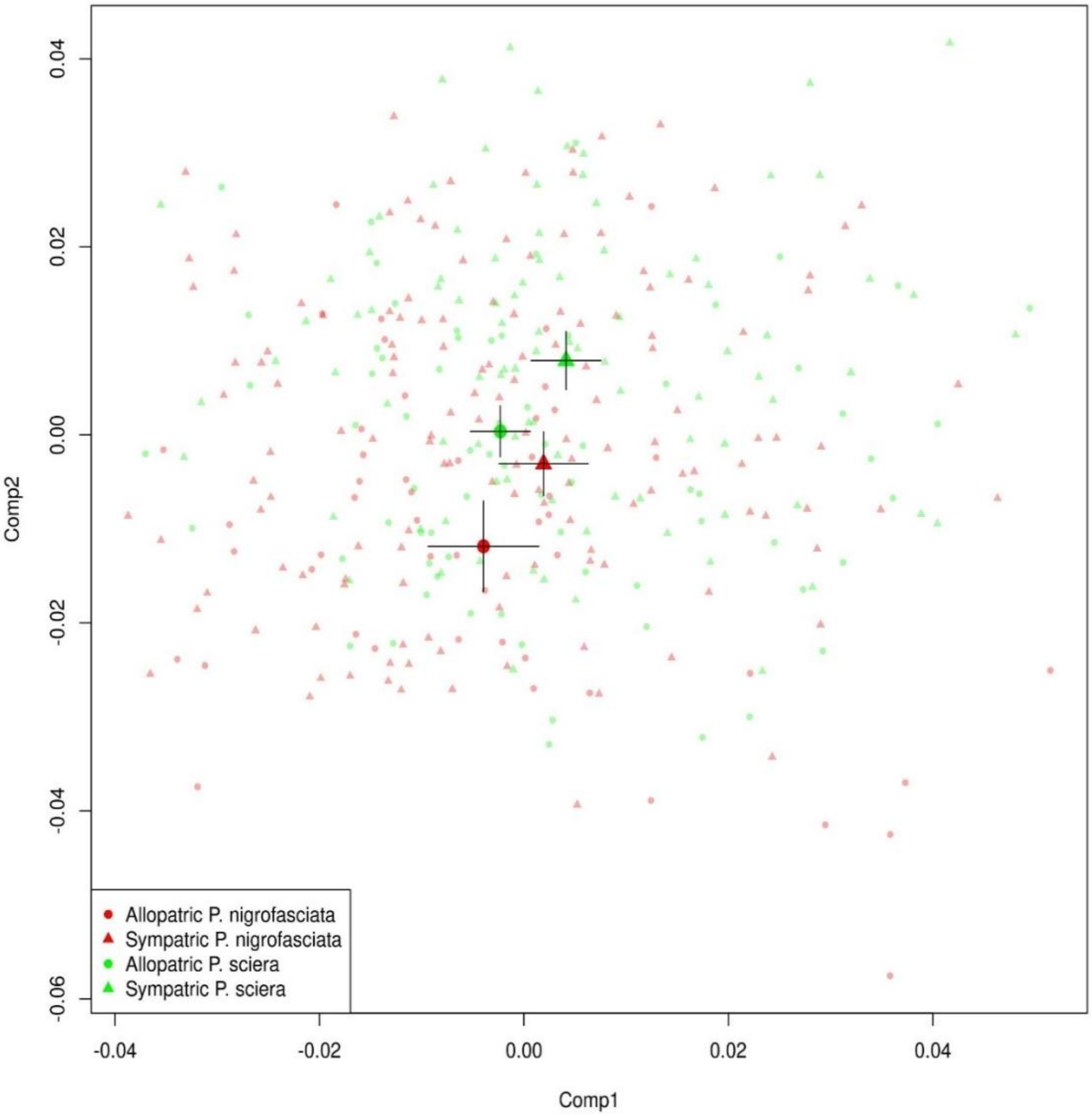

Figure 3: PCA of shape variables. Each point represents a single specimen's species and whether it was in sympatric or allopatric populations. The larger points with bidirectional error bars represent the means and 95% confidence intervals of each species in each type of population. The red shapes represent *P. nigrofasciata* and the green shapes represent

P. sciera. The triangles represent individuals in sympatric populations while the circles represent individuals in allopatric populations.

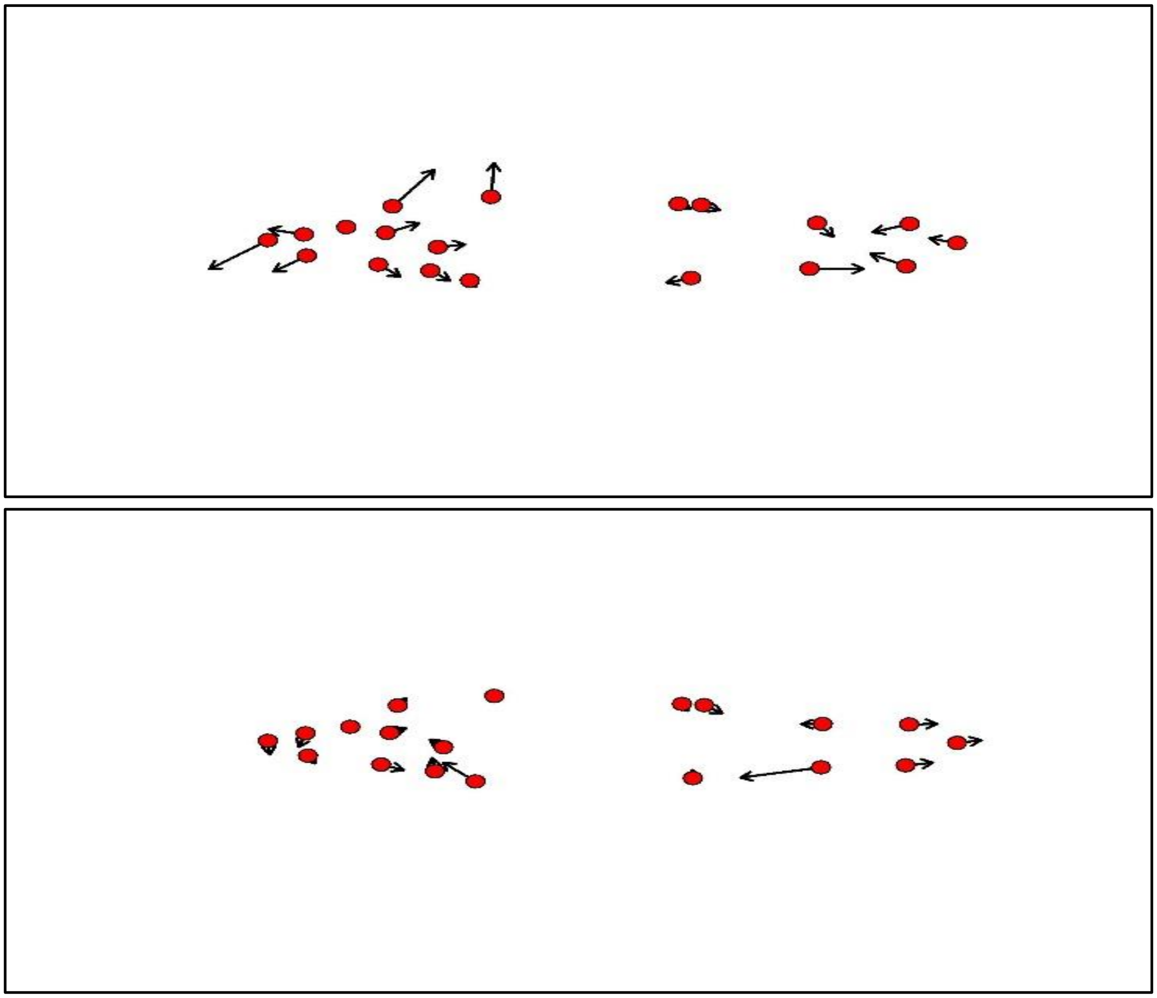

Figure 4: Shape variation displayed between species and treatment. The image on top depicts the changes that occur moving from *P. sciera* to *P. nigrofasciata* (allopatric and sympatric populations combined). The image on bottom depicts the changes that occur moving from allopatric populations to sympatric populations (both species combined). The magnitude and direction of change that occurs at each of the landmarks is represented by the black arrows.

CHAPTER IV: DISCUSSION

The larger difference seen in the results of the MANOVA (Table 3) between the species was more expected because they are in fact different species. Even though these species look similar with similar colorings and patterns and are often hard to separate from one another, they are still distinct species (Page, 1983). They would therefore be expected to display differences that distinguish them from similar species. The second biggest difference (that seen among the sizes of the individuals) was also expected because this represents the changes that the fish undergo as they grow. In order to better control for size, individuals were selected from a narrow range, first based on approximate size then weight, ranging from 0.42-4.55 g. This difference seen can be related to allometry, which refers to how certain areas of the body grow at different rates compared to others, resulting in body proportions changing.

Because the MANOVA values for the interaction between treatment and species was much smaller than the other values, these results were less important. However, this is not unexpected since species and allometric differences are expected to dominate. This is supported by the PCA since the plot suggests that the species change similarly. The change in body morphologies (Figure 4) demonstrated deeper heads, longer snouts, and a shortening of the caudal peduncle when moving from *P. sciera* to *P. nigrofasciata*. According to Page (1983) there is a strong relationship between darter morphologies and the habitats they are found in, meaning that the habitat is often one of the main factors driving the fish's morphology. These two species are both midwater species that have relatively fusiform bodies and are often found in gravel-like raceways and riffles with deep caudal peduncles (Page, 1972; 1983).

When the species are in sympatry, the morphologies changed by expanding the caudal peduncle. This could be explained by the species expanding their niche to include habitats with different current velocities, specifically faster flowing habitats. The pattern of the caudal peduncle changing in association with the flow would support the hypothesis that character displacement drove the differences seen in this study. Several examples from the literature showed that fish species that were associated with faster flowing waters were seen to have larger or expanded caudal regions. Salmon and brown trout (*Salmo salar* m. *sebago* and *Salmo trutta* m. *lacustris*, respectively) that were raised in faster flows had larger caudal fins than their slower flow counterparts (Pakkasmaa & Piironen 2001). These larger fins would allow the fish to maintain their position in the flow better instead of being swept downstream away from their habitat (Riddell & Leggett 1981). Kerfoot Jr. and Schaefer (2006) found that sculpin in habitats with slower flow velocities had wider caudal peduncles as well as deeper and wider body depths. The differences in the pressures from the environment on the fish could have caused their morphologies to change as they shifted to meet the requirements placed on them by the habitat (Kerfoot Jr. & Schaefer 2006). Haas et al. (2015) found that *Cyprinella venusta* in habitats with higher mean annual run-off shifted toward more streamline body forms with slender bodies and caudal peduncles. This body shape would allow them to better handle the faster flows associated with the higher run-off levels (Haas et al., 2015). In brook char, *Salvelinus fontinalis*, individuals that used more littoral or pool habitats, were found to have shorter, dorsoventrally expanded caudal peduncles (Samways et al., 2015). Because the fish did not have to compete with the higher water flow, the change to have a larger or expanded caudal peduncle was selected for in order to increase the species's fitness. This would give them better control over maintaining their position in the water column as well as the habitat they are found in. If the caudal region were more reduced, the fish may struggle to stay in its habitat or lose its position in the water column. This could prevent it from capturing prey or escaping predators.

Conclusion

The results of this study support Gause's Principle in that co-occurring species will divide habitat use at small scales, such as within the same riffle habitat, due to competitive interactions (Greenberg, 1988). Even though the difference between treatment and species was smaller compared to the other categories in the MANOVA and deemed less significant, these results still showed that these two species change differently when in sympatry vs. allopatry. These results could possibly explain why the southeastern United States is a biodiversity hotspot with high levels of endemism, especially in fish species. As morphologies changed to allow for the expansion of niches, similar species would have diverged into new species so that these similar species would be less likely to experience the effects of competition.

Future studies should seek to examine differences between individual drainages instead of major drainages systems to see if allopatric and sympatric individuals differ at a finer scale. If differences are seen at a finer scale, this could add support to why the southeastern United States have such high levels of biodiversity in fish species.

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