Ecomorphological analysis of cyprinids in the Mobile Bay Basin

by

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Abstract

Ecomorphology is the study of variations in morphology and how it relates to organisms' various life histories or ecologies. Phenetic packing/dispersal studies analyzes morphologies of community members and sees how the phenotypes overlap. Because an organism's ecology is often manifested in morphological traits, phenotypic overlap can act as a proxy for ecological or niche overlap. The cyprinids of the Mobile Bay Basin are both diverse in species and in their morphologies which makes them an ideal group for a phenetic packing/dispersal studies. Average nearest neighbor distance statistics are utilized in these studies to determine the degree of packing/dispersal. Packing remained relatively the same throughout all communities examined in the Mobile Bay Basin. However, when introduced species were added to the cyprinid communities, the introduced species from elsewhere in North America showed high overlap with native species in Mobile Bay Basin. These species have yet to successfully colonize the basin. The overlap suggests that the introduced North American species are being outcompeted by native species. Introduced Asian cyprinids have successfully colonized the basin and show no overlap, indicating potentially that they occupy niches not filled by native species. Therefore, morphology can aid in predicting whether introduced species will successfully invade a new ecosystem.

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General Introduction

Ecomorphology is the study of variation in morphology between individuals, populations, species, and communities, and how it relates to their ecology (Leisler and Winkler, 1985). The majority of ecomorphological studies have been conducted using vertebrates as the study organisms. The long list of vertebrate types utilized in this type of research include birds (Rickleffs and Travis, 1980), bats (Findley, 1976), rodents (Brown, 1975), lizards (Rickleffs, 1981), and to a lesser extent, fishes (Winemiller, 1991). With the knowledge gained from this extensive ecomorphological research on vertebrates, inferences can be made about an organism's ecology based upon its morphology. Therefore, morphospace can be used as a proxy for ecospace. When two species overlap in morphology, they could potentially be competitors. If the two species are competing, divergent natural selection may

cause shifts in the phenotypes to limit the amount of competition (Day et al., 1994).

Phenetic packing/dispersal studies use morphospace as a proxy for ecospace across multiple species in a community, to potentially predict which ecological factors are structuring a community, and to examine the interplay among various phenotypes in a community. Phenetic packing occurs when a species is found within the confines of the community's shape space (Findley, 1973), whereas phenetic dispersal occurs when a species' morphology lies outside of community morphospace (Gatz, 1979). Specialists tend to be more dispersed, because theyhave evolved unique morphological traits in their specialized feeding or microhabitat choices (Ricklefs and Travis, 1980). This pattern can cause a community to be more dispersed than packed. Generalists tend to possess the same morphological features, and cause a community to be more packed than dispersed (Ricklefs and Travis, 1980).

When introduced species are added to a community, their position in morphospace can be used to potentially predict competitors within the recipient community. Introduced species with morphology that overlaps native species may compete with native species and be excluded from the community (Kirichenko et al., 2013), or the introduced species may out-compete the native species and extirpate it from the community. If there is no morphological overlap between introduced species and any of the native species, the introduced species could experience competitive release and thus be more likely to establish a population within the community. This thesis aims to address the lack of research involving fish in phenetic packing studies as well as utilizing morphology to predict the successfulness of introduced species in new ecosystems.

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Chapter 1: Phenetic Packing Revisited With Modern Techniques:

A Case Study with Minnows in the Mobile Basin

Abstract

The morphologies of species in a community can reveal potential abiotic and biotic factorsthat structure the community such as competition, predator avoidance, niche filtering, and disturbance. Phenotypes of organisms can be phenetically packed into a tighter area of morphospace, or phenetically dispersed to occupy a larger area of morphospace. Studies on phenetic packing/dispersal in vertebrates were common in the 1970s and 80s, but few used fish as study organisms. Recently-developed techniques in geometric morphometrics offer a precise way to analyze the shapes of fishes within a community. The present study used geometric morphometrics to reveal that cyprinids in the Mobile Basin maintain a steady rate of phenetic packing/dispersal across 21 communities, regardless of species richness,and that pool habitats were more packed than riffles.

Introduction

Competitive interactions among species arise when species utilize the same limited resource, which includes habitat, food resources, mates, or breeding sites. Species can avoid competition by expanding or limiting aspects of their niche, such as becoming a generalist or specialist regarding feeding and microhabitat use, foraging at different times, and establishing complementary breeding habits (Chesson, 2000). The latter are examples of Diamond's (1975) assembly rules that state competition should cause limited niche overlap between species and prevent certain species from co-existing. When a species' niche shifts, it is often reflected in a change in morphology. This morphology-niche relationship has been studied extensively in vertebrates, including birds (Ricklefs and Travis, 1980), bats (Findley, 1976), rodents (Brown, 1975), lizards (Ricklefs, 1981), and fishes (Winemiller, 1991). Interspecific competition typically causes morphological dispersion among competitors, which may translate into divergent resource use, and thus reduced use of shared limited resources.

Phenetic dispersal results in varying phenotypes across species in a community (Gatz, 1979). For example, sticklebacks showed non-directional selection until another species was added to the environment, at which point selection then favored the individuals of both species that were the most dissimilar morphologically from each other (Day et al, 1994). The counterpoint of phenetic dispersal is phenetic packing, in which morphological space is constrained by environmental selection pressures (Findley, 1973). For example, two insectivorous bat sub-communities were found to be tightly packed in terms of their wing aspect ratio, indicating that vegetation cover, was more important than competition in structuring the communities (Chrome and Richards, 1988). The above insights into ecology and evolution were all derived using morphospace as a proxy for ecospace.

Morphospace can be used as a proxy for niche-space, so morphology of species can offer some, albeit limited insight, into how communities are structured. Researchers have paid limited attention to phenetic packing and dispersal since the 1970s-1980s, and none have used modern techniques like geometric morphometrics, which can preserve shape better than traditional morphometrics thus allowing for a better, more precise morphospace analysis. Finally, few studies have utilized fishes as their study organisms (Gatz, 1979 and Winemiller 1991). My research utilized this least-used vertebrate taxon, fish, to study phenetic packing/dispersal, and applied the technique of geometric morphometrics. Cyprinidae is a very diverse and speciose fish family that could provide insight into the rates of phenetic dispersal and packing in fishes, as well as the ecological factors that structure aquatic communities. This research also may provide broad implications for the formation and maintenance of fish communities in general.

Interspecific Competition in Communities: Phenetic Packing and Dispersal

Phenetic packing and dispersal both can occur when a species is added to a community (Findley, 1973; Gatz 1979). Species may join a community through dispersal or migration (Shurin, 2000), when they evolve within the community through ecological speciation (Nosil, 2012), or when they are introduced through anthropogenic means (Ricciardi, 2001). Phenetic packing and dispersal differ in terms of the positions that species occupy within morphospace, after they assimilate into new communities. Morphospace indicates where species occur in a continuum of body shapes (or varying body part morphology such as arms or limbs), based upon an average overall shape for all organisms included in the analysis. Visualization of morphospaceis usually generated using principal components analysis (PCA, Fig. 1; see also chapter 2). The PCA displays shape-space on a 2D plane, based upon the components of shape that differ most among species. Phenetic packing occurs when the species is added inside of the pre-existing morphospace (Findley, 1973) whereas phenetic dispersal occurs when the species is added to the periphery of morphospace, thus expanding the pre-existing morphospace occupied by members of the community (Gatz, 1979; Winemiller, 1991).

Studies of actual phenetic communities versus synthetic (hypothetical) communities have compared the average nearest neighbor distances (NND) among individuals in them, using synthetic communities as a null model (Rickleffs, 1981). This test reveals whether natural communities differ from what one would expect to find in a random assemblage of species. The average NND indicated whether a community is phenetically dispersed or packed.

Patterns in Phenetic Packing

It is important to highlight ecologically relevant morphological traits. Selection of irrelevant traits would not provide information on how these phenotypes interact between species. For example, in birds, it was found that phenetic packing occurred with some traits, but not with all (Karr and James, 1975). For ecologically relevant traits, there is a limit to the amount of phenotypes that can be "packed" into an ecosystem before new variations most be derived (Schum, 1984). After 20 species with similar phenotypes occur sympatrically, new species must be added to the periphery of morphospace, and this variation in morphology serves to limit interspecific competition (Brown, 1975). In addition, higherrates of packing occur in smaller assemblages, because fewer species can share resources (Schum, 1984), and generalists tend to be more packed than specialists. They possess similar morphological traits that allow them to feed on diversefood sources and persist in multiple habitats (Rickleffs and Travis, 1980). When morphospace becomes tightly packed, species specialize, and are added to the periphery of the morphospace, known as phenetic dispersal (Fenton, 1973; Findley 1973; 1976).

Patterns in Phenetic Dispersal

Phenetic dispersal states that as species are added to an assemblage, morphospace should expand (Schoener, 1974Ricklefs and O' Rourke, 1975; Ricklefs and Travis 1980). Communities with more specialists tend to trend more towards dispersal than packing, because specialist often must evolve unique morphological features to cope with their specialization (Rickleffs and

Travis, 1980). Once an assemblage reaches a certain level of richness, morphospace must expand to reduce competition, with the threshold for this to occur being between 20 and 25 species (Schum, 1984). Once species richness increases beyond this point, species should spread out further along the vectors of the first few principal components (Schum, 1984). However, there remains only a single study using fish that showed no increase in packing or dispersal in more richer assemblages Winemiller, 1991). There, the rate of packing remained relatively constant over a range of species found in the communities studied (Winemiller, 1991).

Effects of Phenetic Packing and Dispersal in Shape Space

Theory predicts that phenetic packing will limit morphospace, thus in highly packed communities, average NND should be small (Findley, 1973). A larger NND and an expansion of morphospace indicate phenetic dispersal (Gatz, 1979). Several factors may influence the way morphospace behaves in more or less speciose systems. If competition shapes community structure, then an overall expansion of shape space would be expected (Juliano et al., 1990). Alternatively, if abiotic disturbance affects shaping community structure, then morphospace would be expected to expand, as only stable environments allow for greater niche overlap (Findley, 1973). In this latter case, both competition and disturbance within a community would both likely influence phenetic dispersal.

There are several ecological factors that could influence a community to be more phenetically packed.Predation could allow for more species to pack into a community by keeping the relative abundance of competitors low (Vandermeer et al., 2006). Niche filtering occurs when the abiotic factors in an environment force sympatric species to converge on a certain lifestyle or body shape through strong selection (Mouillot, 1997). Niche filtering also causes a restriction in overall shape space, because of strong stabilizing selection towards a common morphology. Specialists typically evolve specific external traits for specialization in certain trophic guilds or breeding habits, causing an expansion in morphospace (Rickleffs and Travis, 1980). Therefore, if a community has many specialists, then the overall shape space would expand.

Niche filtering can also exclude new species from invading a community if the new species lack the prerequisite features needed to survive the constraints that the environment places on the species already in the community. For example, high soil acidity could exclude certain species of plants that cannot tolerate low pH (Mouillot, 1997), just as riffles could exclude invasive stream fish not adapted for endurance swimming. Fast flow speed in riffles should select for more streamlined morphologies, thus leading to the phenetic packing. Species in pools, however, are subject to other biotic and abiotic factors that could affect morphospace such as disturbance and predation. Physical disturbance, particularly the rise and fall of water levels in pools during storms, could force a larger area of morphospace than in riffle habitats, because more disturbed communities tend to be more morphologically diffuse than more stable systems which allow greater resource overlap (Findley, 1973).

Relationship Between Morphology and Ecology

An organism’s behavior is often related to its morphology in that certain physical traits can relate to trophic level and preferred habitat (Schoener, 1974). In fish, diet is affected by the position of the mouth. Superior mouths indicate that fish feed from the upper portion and surface of the water column, whereas terminal mouths indicate a more generalist diet, and inferior mouths designate that the fish feed from the substrate or benthos (Winemiller, 1991). The mouth to body size ratio of a fish also can indicate whether it is carnivorous or omnivorous/herbivorous, as fishes with larger mouths tend to be carnivores (Costa and Cautadella, 2006). Fin position and body depth also indicates whether the fish burst swims or endurance swims (Costa and Cautadella, 2006), with a deeper body and higher fin position typically indicating burst swimming associated with lentic-like habitats (stream pools, backwaters, swamps, and lakes). In contrast, shallower body and lower fin position indicate that the fish evolved for endurance swimming, and is associated with faster-flowing habitats like streams or rivers (Winemiller, 1991). The height of the caudal peduncle also indicates whether the fish is a carnivore, omnivore, or herbivore; predators tend to have a narrower caudal peduncle indicating a fast swimmer, herbivores have a broad peduncle (slower swimmer), and omnivores have an intermediate form (Costa and Cautadella, 2006). In addition, fin placement also affects fish agility and maneuverability of a fish. Fish in more complex environments such as reefs have higher pectoral fins than fish in less complex habitats, which aids inmaneuverability andin predator avoidance (Costa and Cautadella, 2006).

Associations between morphology and ecology have been demonstrated in several vertebrate taxa. For example, bat wings, ears, and cranial structure affect their feeding behaviors and habitat use (Fenton, 1973; Findley 1973, 1976), foot size and shape affect field mouse *Peromyscus* habitat prefernce (Brown, 1975), and beak and wing shape affect bird habitat use and feeding behaviors (Ricklefs and Travis, 1980).

Novel Approach to Examining Phenetic Packing versus Dispersal

Previous phenetic packing and dispersal studies have used traditional morphometrics to describe patterns; however, this method represents only a subset of shape measurements and does not fully express overall shape (Zelditch, 2012). A more comprehensive approach to quantifying shape is geometric morphometrics. This methoduses a series of homologous landmarks that better characterizes organism shape, than do traditional methods (Zelditch, 2012). By utilizing geometric morphometrics for the first time here in a phenetic packing and dispersal analysis, valuable insight was gained into community structure based upon morphospace.

Study Organisms and System

The southeastern United States hosts more freshwater fish species than anywhere else in North America north of Mexico, with Alabama having more diversity of species than any other state besides Tennessee (Warren et al., 2000). Many species are in the Order Cyprinformes, a large monophyletic group of freshwater fishes which includes the minnows, chubs, suckers, and carp (Mayden et al., 2009). This large group plays an important ecological role in all freshwater habitats in Alabama, and it composes a large part of fish assemblages. Cyprinidae (minnows) is the largest family within the order, and a major portion of Alabama fish species diversity and abundance occurs within this family. Cyprinids display a wide variety of body shapes and sizes. This variation in phenotype could facilitate understanding of the ecological forces structuring these cyprinid communities. The purpose of my thesis research to explore the factors that shape cyprinid communities based on body shape, in terms of phenetic packing vs. phenetic dispersal.

The Mobile River Basin is one of the most speciose river basins in temperate regions worldwide, with 54 species of native minnows and 6 introduced cyprinids (Warren et al., 2000). It consists of 7 major tributaries that all flow roughly north-south, thus minimizing latitude as a factor: the Coosa, Tallapoosa, Alabama, Tombigbee, Black Warrior, and Cahaba rivers as well as the Mobile-Tensaw Delta (Mayden et al., 2009, Fig. 3). The Mobile Basin extends across parts of Mississippi and Georgia and most of Alabama. The northern and southern sections of the basin are separated by a stark change in habitat delineated by the Fall Line. North of the Fall Line are high-gradient rocky streams, and to the south are low-gradient sandy streams (Boschung and Mayden, 2004). This strong contrast of habitats could also force some interesting effects on morphospace in the rivers that cross the Fall Line. With multiple habitats provided by the Fall Line, there could be an overall expansion of morphospace, because more niches are available for fishes to exploit than in a river basin lacking a major physical change in river structure at its center.

Study Objectives

The main objectives and associated hypotheses encompassed by the present study were as follows:

1) Determine the degree to which the river systems of the Mobile Bay Basin are phentically packed or dispersed as more species are added to the river sections downstream.

Hypothesis: Morphospace occupies a larger area as cyprinid species are added downstream, thus causing phenetic dispersal.

Null Hypothesis: All sections of the rivers are equally packed or dispersed, and do not differ in morphospace from randomly-generated (synthetic) communities.

2) Analyze variation in morphospace between stream microhabitats (riffles and pools) to determine the potential underlying ecological mechanisms structuring cyprinid assemblages.

Hypothesis: Riffles are more phentically packed than are pools due to niche filtering.

Null hypothesis: Cyprinid morphospace does not vary significantly among microhabitats.

Methods

Twenty-five photographs of the 54 native species of cyprinids within the Mobile Basin were taken, and 18 homologous landmarks were placed upon the photographs according to Armbruster (2012). All photographs were taken from specimens in the Auburn University Museum of Natural History (AUMNH). Lots containing specimens were selected throughout the species' home range within the Mobile Basin to maximizeintraspecific variation within the species tested. The most suitable specimens for geometric morphometrics within the lots (categorized as specimens that were well-preserved, with straight bodies, and large enough to be adult size), were then chosen for photographing. By using museum specimens collected over a period of 60 years, and their distribution records along with records described in Boschung and Mayden (2004), this study avoids a "snapshot" view of the communities. The combined data from AUMNH and Boschung and Mayden (2004) records allow for the communities to be characterized based on nearly a century of collection data with records delving back into the 1920s. In contrast, past phenetic packing studies have relied on short periods of collecting to characterize the communities found in certain areas. These collections could miss important species in the communities or pick up transient species that are not a true component of the long-term fauna in an area.

All specimens chosen were between 35 and 60mm SL to assure that only adults were used in the analysis. Landmarks were placed using the program tpsdig2 (Rohlf, 2006). A Generalized Procrustes Analysis (GPA) was applied to the data to align the specimens onto the same plane and scale. The data were then subjected to a PCA to produce a multivariate description of shape space. The 25 data points per species were averaged to produce one data point for each of the 54 species. The GPA, PCA, and consensus points were calculated in the program MorphoJ (Klingenberg, 2011). Average NND was conducted using R for the average data points for each species (R Development Core Team, 2008).

The above methodology was applied to the upstream, median, and downstream portions of the examined rivers (see Fig 3). The rivers were divided into fifths based on river mile. The first fifth was categorized as the upstream portion, the third fifth as the median portion, and the final fifth as the downstream section. By selecting these fifths, an arbitrary break between communities was established. This was applied to the main stem and tributaries of each river section examined, so that the cyprinids in the main stem and tributaries acted as a meta-community.

The natural communities were tested against a null model of randomly-generated communities with the same species richness. The synthetic communities were limited in their PCA values based upon the highest and lowest PC scores for actual fish in the basin, and were created using the random data generator in Excel, to determine if there was any biological significance to the PCA data. If a community displayed phenetic dispersal, average NND should be significantly smaller than that of the synthetic community; in contrast, if a community displayed phenetic dispersal, average NND should be significantly larger than the synthetic community. An ANOVA was used to determine if there was a significant difference among the headwaters, midstream, and downstream portions across river basins, and then a Tukey’s post hoc test was utilized to determine where differences among means resided.

The above methodology also was applied to the species in the pools versus riffles of each river. As stated above in the Introduction, I predicted an increase in phenetic dispersal within communities from the headwaters to the downstream portions of the Mobile Basin, that phenetic packing occurs in riffle microhabitats, and phenetic dispersal occurs in pool habitats.

Results

Cyprinid richness increased across all rivers in the Mobile Basin from the headwaters to the downstream areas (Table 1). When average NNDs of natural and synthetic communities were compared, they were significantly different ($p = 0.0291$), indicating that actual assemblages were not randomly structured. PC1 was primarily described by body depth and described 25.1% of the variation. PC2 was primarily described by head shape and size and described 18.0% of the data.

Average NND for the upstream assemblage was 0.0087, and midstream and downstream assemblages were0.0077 and 0.0074, respectively. Despite a trend in the data showing average NND decreasing from upstream to downstream (Fig. 4), the results were non-significant ($p = 0.1038$). Upstream assemblages differed significantly from downstream assemblages along PC1, whereas upstream and midstream assemblages did not differ along this vector ($p = 0.5125$). Midstream did not differ significantly from downstream along PC1 (Table 2).

Average NND in pools was 0.0094, whereas average NND in riffles was 0.0130 (Fig. 5 and Fig. 6). The difference between microhabitats was non-significant, possibly due to limited sample size ($p=0.362$).

Discussion

Phenetic Packing Along a River Continuum

In my study, the natural fish communities were significantly different from the synthetic communities, which suggests that processes affecting phenetic packing of cyprinids in the Mobile Basin are not random,but rather have biological significance. This significance suggests that these assemblages adhere to some form of assembly rule. Upstream communities were significantly different from downstream areas along PC1 (the vector that largely accounted for change in body depth), but were not significantly different when analyzed by average NND. Examination based upon PC loadings instead of average NND may provide more information, as fewer traits are being considered per analysis. This aids in finding traits that are ecologically significant since analyzing PC1 and PC2 combine more traits than just a single vector.

The rates of packing held constant throughout the length of the rivers according to the average NND, which was similar to Winemiller's (1991) findings. There could exist an ideal phenotypic distance from nearest neighbors. If interspecific competiton was important, this ideal distance could limit competitive interactions while promoting more morphologically dissimilar species into the community. The distance itself may evolve as a by-product between two or more competing, co-evolving species. Once aspecies has reached a certain morphological and ecological distance, the selection for differing phenotypes may be relaxed, thus leaving an ideal competitive distance between it and sympatric species.

Alternatively, disturbance fromseasonal changes may disallow for much niche overlap, as less stable ecosystems allowed for fewer species (Findley, 1976). Climatologically stable ecosystems allow for more complex interactions and niche overlap to evolve. These disturbances to regularity due to seasonality in the temperate zone could be preventing much ecological, and subsequently morphological, overlap among cyprinids. Throughout the Southeast, recent reservoir and dam construction have greatly impacted flow regimes in rivers as well, adding to the instability of abiotic factors. Stream fish adapt to this type of anthropogenic habitat change rapidly through changes in morphology (Franssen, 2011). Also, exotic species such as *Cyprinella lutrensis* breed regularly with other cyprinids, thus inserting their genes into the gene pool (Devivo and Freeman, 1995). Changes to the gene pool resulting from the above factorscould change the overall phenotypes of native cyprinids.

Despite the non-significance of average NND among the different sections, the values for the PC1 vector were significantly different from upstream to downstream. PC1 was the difference in body depth, which indicates habitat preference with body depth increasing in downstream areas. Deeper-bodied fishes prefer more lentic areas such as pools or lakes, and more shallow-bodied fish prefer lotic habitats such as riffles or runs/raceways (Cureton and Broughton, 2014). As more species were added to the river systems (Table 1), the PC1 vector was extended as species added down-stream had deeper bodies. Thissuggests a different preference for habitats with slower flow speed, which characterizes downstream habitats.

Species Packing in Pools vs. Riffles

Despite the non-significant relationship between pools and riffles, there was a general trend for pools to be more packed than riffles. This could be a product of having too small of a sample size (n=7). This area merits more study. Pools may be more packed, simply because the number of species in pools outnumber the species in riffles within the Mobile Basin (Table 2). Riffles are more energetically stressful microhabitats in which to live, because faster flow in riffles causes metabolic pressure on fish (Rand and Hinch, 2011). Because riffles are energetically taxing, there could be fewer species present than in pools, leading to less packing in riffles. This is surprising, however, because lower diversity typically correlates with higher rates of packing (Schum, 1984).

Conclusion

The relationship between packing/NND and position along this river continuum, while non-significant, showed a general trend towards packing as species are added in downstream areas, in support of previous studies. This could be a product of having too small of a sample size and needs more study in the future to determine if this trend is biologically significant or not. All of the rivers examined approached Brown's (1975) number of species for maximum packing, but never exceeded the number that forces divergence in phenotypes to dilute competition. PC1 scores varied significantly from upstream to downstream. The PC1 vector (mainly body depth) increased from upstream to downstream, indicating that habitat differentiation and niche segregation is important to building cyprinid communities in the Mobile Bay Basin.

To determine if the relationship among pools and riffles is biologically significant, a greater sample size of cyprinid communities in pools and riffles is needed in future research. In addition, the actual space utilization of species needs to be better examined. Some species of minnows are occasionally found in riffles, but are likely transients. The study could be expanded to other basins as well, perhaps some with larger communities of cyprinids. The relative packing or dispersal in riffles vs. pools could also be explored in greater depth with other families such as percids in North America, or could be explored with larger faunas in tropical rivers. Other families of fish have exploited available niches in riffles, in ways that North American cyprinids have not, thus making relative packing in pools vs. riffles possibly taxon-specific.

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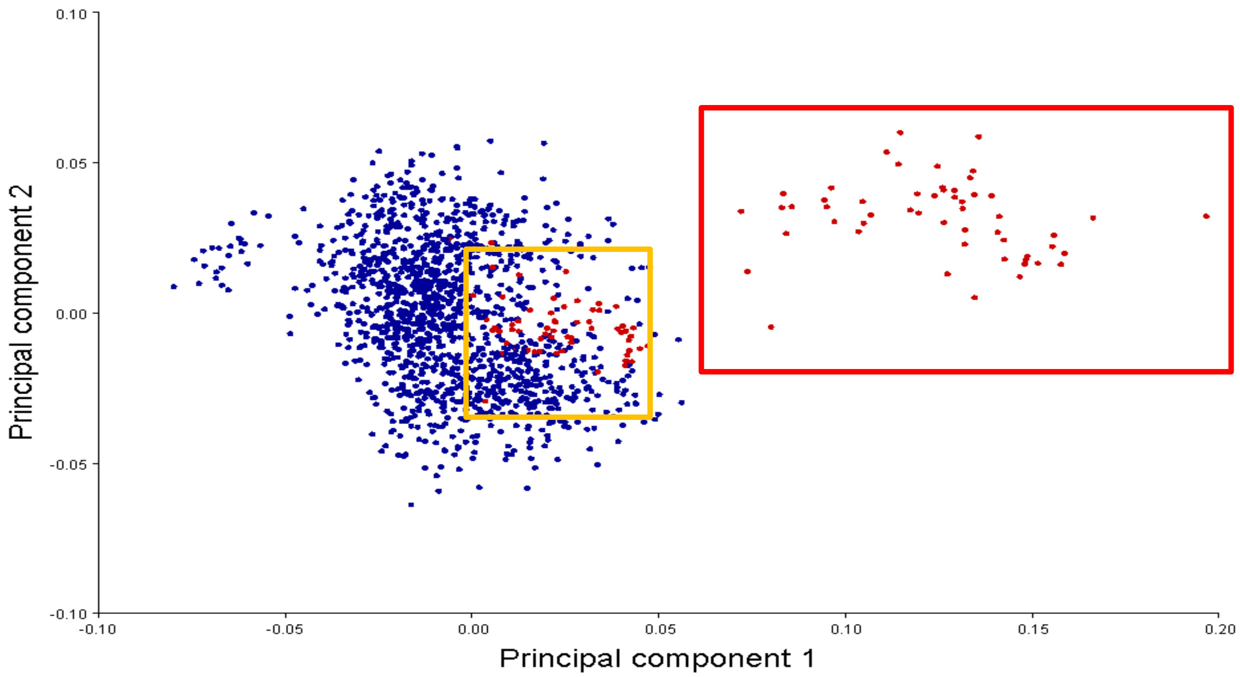

Figure 1: Principal Component Analysis of 25 individuals in each of 60 species of native (blue) and introduced (red) cyprinid fishes in the Mobile Basin. Species introduced from elsewhere in North America are inside the orange box (*Cyprinella lutrensis* and *Pimephales promelas*) and demonstrate phenetic packing. Species introduced from Asia are in the red box and indicate phenetic dispersal.

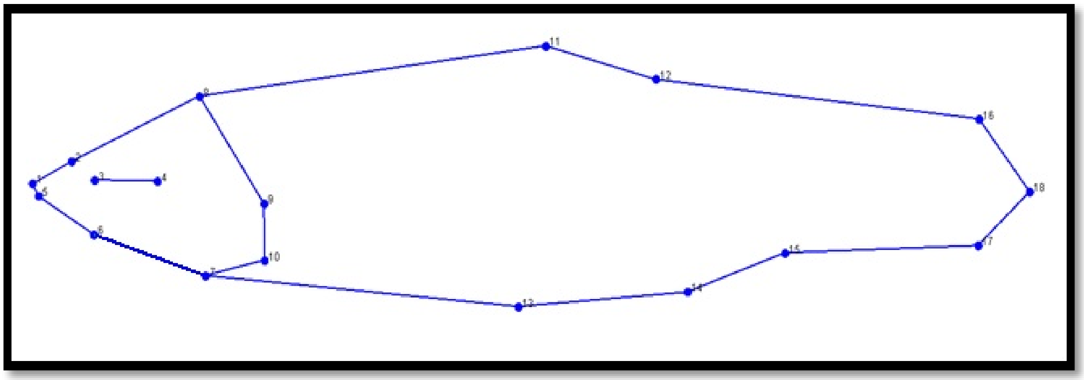

Figure 2: Consensus body shape of all cyprinids in the Mobile Basin. The consensus body shape was generated from the landmark data.

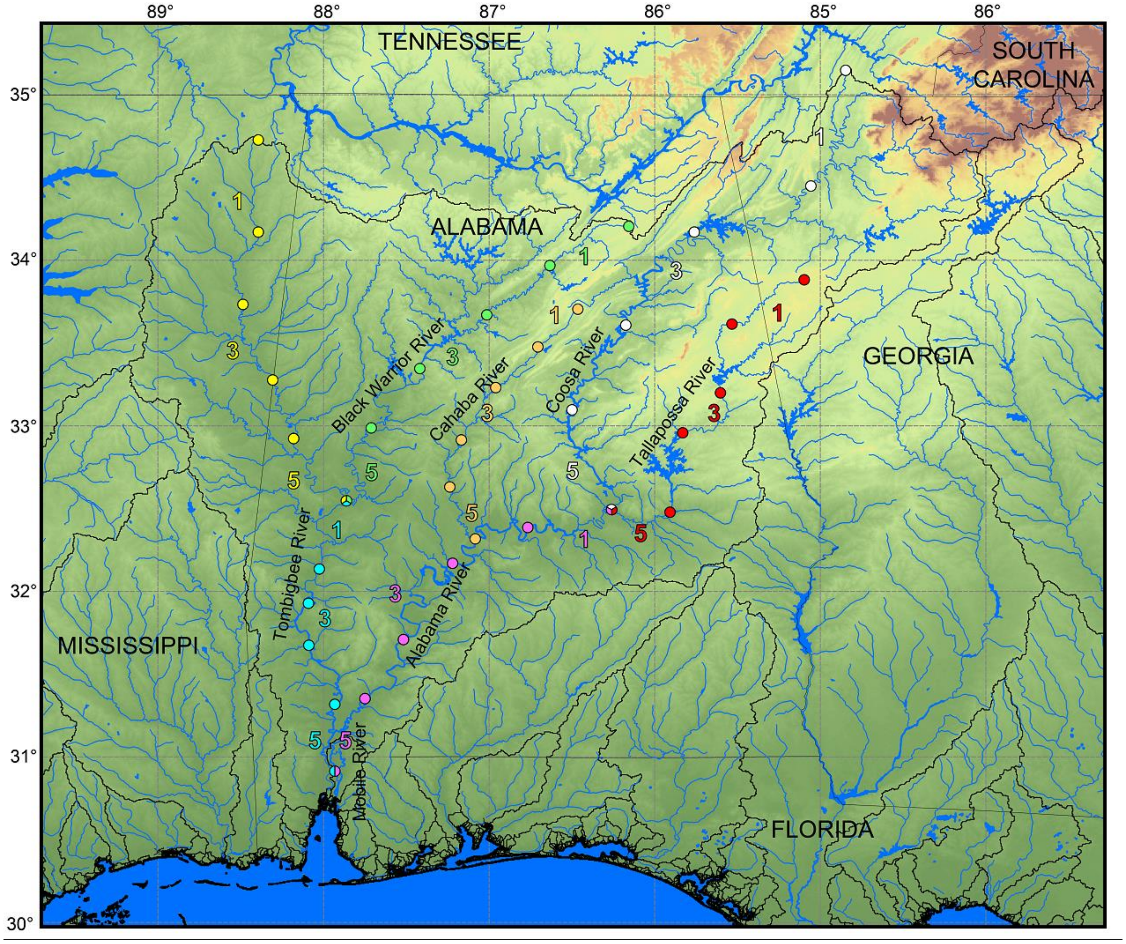

Figure 3: Map of the study area in the Mobile Bay Basin. Colored circles on the map indicate where the rivers were divided into fifths to delineate fish assemblages. Red circlesshow the Tallapoosa River fifths, white circles the Coosa River fifths, orange circles the Cahaba River fifths, yellow circles the Upper Tombigbee fifths, blue circles the Lower Tombigbee fifths, green circels the Black Warrior River fifths, and pink circles the Alabama River fifths.

Table 1. Locations of all cyprinids within the Mobile Basin by drainage split, into upper (U), middle (M), and lower (L) fifths of river sections

	Tallapoosa			Coosa			Cahaba			Upper Tombigbee			Lower Tombigbee			Alabama			Black Warrior			Mobile-Tensas Delata		
	U	M	L	U	M	L	U	M	L	U	M	L	U	M	L	U	M	L	U	M	L	U	M	L
Campostoma oligolepis	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Cyprinella caerulea				X	X		X	X																
Cyprinella callistia	X	X	X	X	X	X	X	X	X		X	X		X			X		X	X				
Cyprinella gibbsi	X	X																						
Cyprinella trichroistia				X	X	X	X	X								X			X	X				
Cyprinella venusta	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Cyprinella whipplei																			X	X				
Hemitremia flammea					X																			
Hybognathus hayi									X	X	X	X	X	X	X			X			X			
Hybognathus nuchalis			X						X	X	X	X	X	X	X	X	X	X			X	X	X	X
Hybopsis lineapunctata	X	X	X	X	X	X																		
Hybopsis winchelli			X		X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X			
Luxilus chrysocephalus	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Luxilus zonistius	X	X																						
Lythrurs bellus		X	X			X	X	X	X	X	X	X	X	X	X	X	X	X			X			
Lythrurus alegnotus																			X					
Lythrurus fasciolaris																			X					
Lythrurus lirus				X	X		X	X																
Lythrurus roseipinnis															X			X				X	X	X
Macrhybopsis aestivalis	X	X	X		X	X		X	X	X	X	X			X	X	X	X			X	X	X	X
Macrhybopsis storeriana			X		X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Nocomis leptocephalus	X	X	X		X	X		X	X	X	X		X	X	X	X	X	X			X			
Notemigonus crysoleucas	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Notropis ammophilus			X			X		X	X	X	X	X	X	X		X	X	X		X	X			
Notropis amplamala		X	X			X		X	X	X	X	X	X	X	X	X	X	X				X	X	X
Notropis asperifrons	X	X		X	X		X	X									X		X	X				
Notropis atherinoides		X	X		X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Notropis baileyi		X	X			X		X	X	X	X	X	X	X	X	X	X	X			X	X	X	X
Notropis cahabae							X	X											X					
Notropis chalybaeus															X			X				X	X	X
Notrpis candidus										X	X	X	X	X	X	X	X	X			X			
Notropis chrosomus				X	X		X	X								X	X			X				

	Tallapoosa			Coosa			Cahaba			Upper Tombigbee			Lower Tombigbee			Alabama			Black Warrior			Mobile-Tensas Delata		
	U	M	L	U	M	L	U	M	L	U	M	L	U	M	L	U	M	L	U	M	L	U	M	L
Notropis edwardraneyi			X			X		X	X	X	X	X	X	X	X	X	X	X			X	X	X	X
Notropis longirostris																		X				X	X	X
Notropis maculatus										X	X	X			X		X	X		X	X			
Notropis petersoni																						X	X	X
Notropis stilbius	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X		X	X				
Notropis texanus		X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X
Notropis uranoscopus			X			X		X	X								X							
Notropis volucellus			X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Notropis xaenocephalus	X	X	X	X	X																			
Opsopoeodus emiliae		X	X			X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Phenacobius catostomus	X	X		X	X		X	X	X								X		X					
Pimephales notatus			X					X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Pimephales vigilax	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Pteronotropis hypselopterus															X			X				X	X	X
Pteronotropis signipinnis															X			X				X	X	X
Pteronotropis welaka															X		X	X						
Rhinichthys atratulus				X	X														X					
Semotilus atromaculatus	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Semotilus thoreauianus	X	X	X			X	X	X	X	X	X		X	X			X	X	X	X		X	X	X

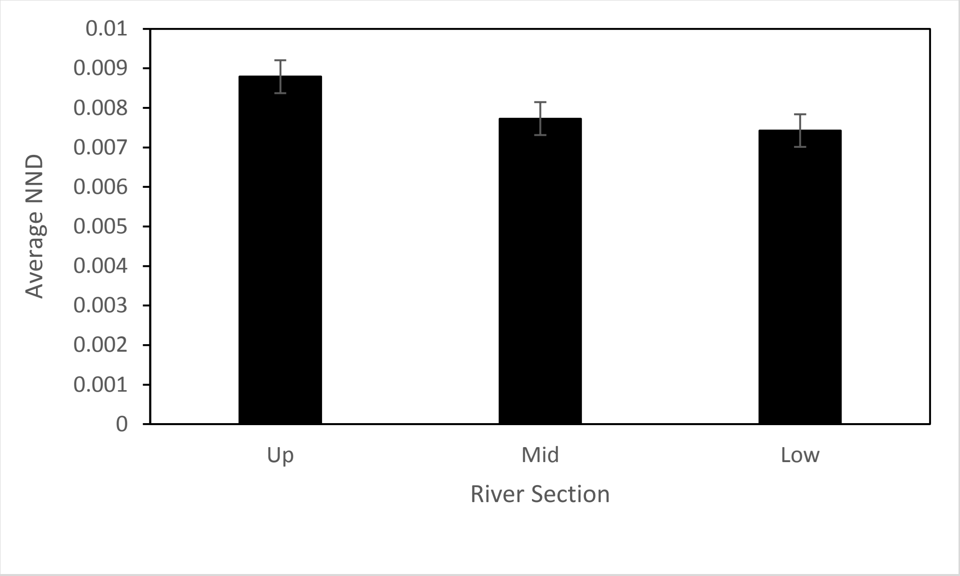

Figure 4: Average NND from upstream, middle and downstream river fifths (n = 7).

Table 2: Significance between river reaches along the PC1 (body depth and head shape) vector. Asterisk indicates significance.

	Midstream	Downstream
Upstream	0.5125	p = 0.0222*
Midstream		p = 0.1897

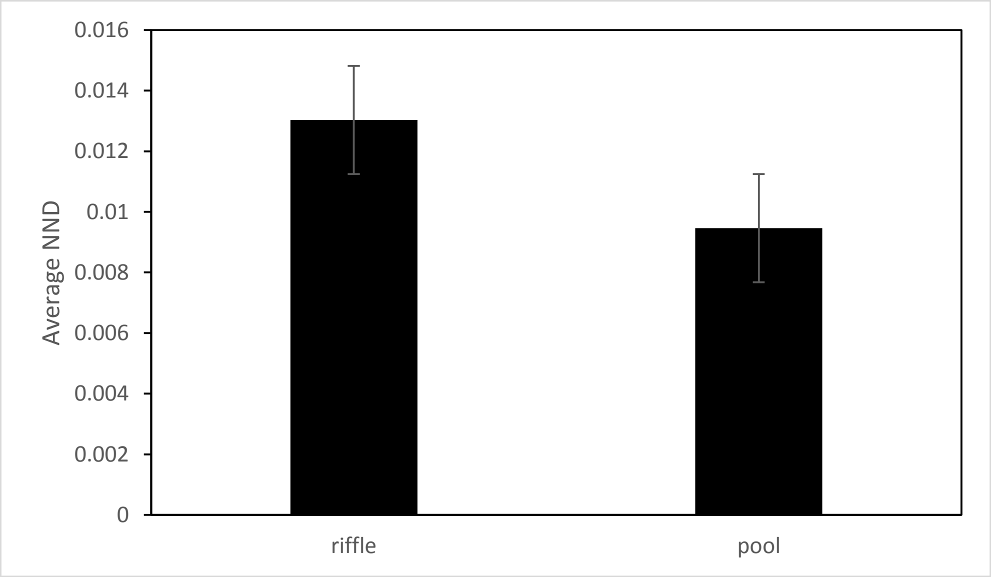

Figure 5: Average NND for riffles vs. pools (n = 7).

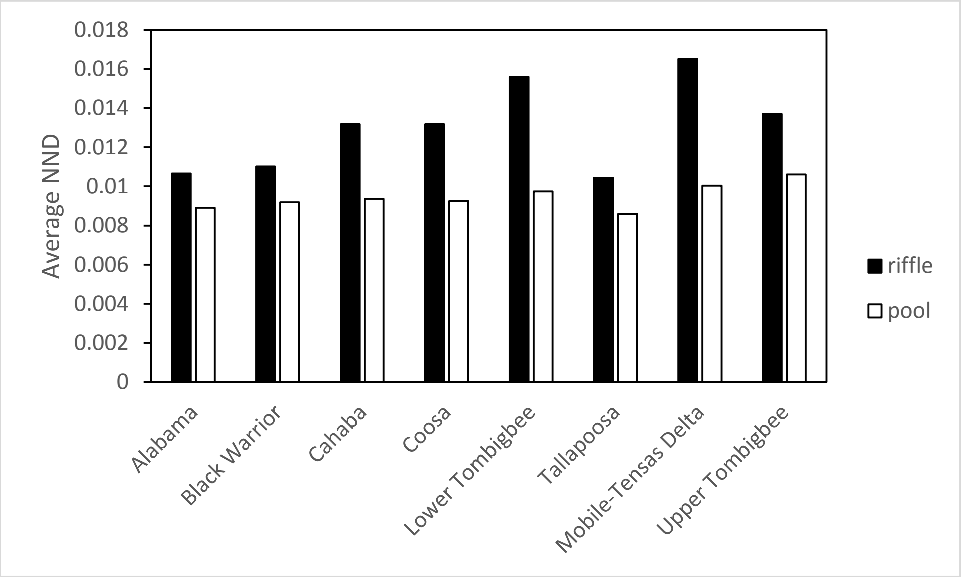

Figure 6: Average nearest neighbor distances for each sub-basin for riffles vs. pools.

Table 3: Species found in pool (P) or riffle (R) habitat based upon river basin.

	Tallapoosa		Coosa		Cahaba		Upper Tombigbee		Lower Tombigbee		Alabama		Black Warrior		Mobile-Tensas Delta	
	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R
Campostoma oligolepsis	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Cyprinella caerulea			X		X											
Cyprinella callistia	X		X		X		X		X		X		X		X	
Cyprinella gibbsi		X														
Cyprinella trichroistia			X		X								X			
Cyprinella venusta	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Cyprinella whipplei													X			
Hemitremia flammea			X													
Hybognathus hayi	X				X		X		X		X		X			
Hybognathus nuchalis	X				X		X		X		X		X		X	
Hybopsis lineapunctata	X		X													
Hybopsis winchelli	X		X		X		X		X		X		X			
Luxilus chrysocephalus	X		X		X		X		X		X		X		X	
Luxilus zonistius	X															
Lythrurus alegnotus													X			
Lythrurus bellus	X		X		X		X		X		X		X		X	
Lythrurus fasciolaris													X			
Lythrurus lirus				X		X										
Lythrurus roseipinnis									X		X				X	
Macrhybopsis aestivalis		X		X		X		X		X		X		X		X
Macrhybopsis storeriana	X		X		X		X		X		X		X		X	
Nocomis leptocephalus	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Notemigonus crysoleucas	X		X		X		X		X		X		X		X	
Notropis ammophilus	X		X		X		X		X		X		X		X	
Notropis amplamala		X		X		X		X		X		X				
Notropis asperifrons	X		X		X						X		X			

	Tallapoosa		Coosa		Cahaba		Upper Tombigbee		Lower Tombigbee		Alabama		Black Warrior		Mobile-Tensas Delta	
	P	R	P	R	P	R	P	R	P	R	P	R	P	R	P	R
Notropis atherinoides	X		X		X		X		X		X		X		X	
Notropis baileyi	X		X		X		X		X		X		X		X	
Notropis candidus							X		X		X		X			
Notropis cahabae						X								X		
Notropis chalybaeus									X		X					
Notropis chrosomus				X		X						X		X		
Notropis edwardraneyi																
Notropis longirostris															X	
Notropis maculatus							X				X				X	
Notropis petersoni															X	
Notropis stilbius	X		X		X		X		X		X		X			
Notropis texanus	X		X		X		X		X		X		X		X	
Notropis uranoscopus																
Notropis volucellus		X		X		X		X				X				
Notropis xaenocephalus	X		X													
Opsopoeodus emiliae	X		X		X		X		X		X		X		X	
Phenacobius catostomus		X		X		X						X		X		
Pimephales notatus																
Pimephales vigilax	X		X		X		X		X		X		X		X	
Pteronotropis hypselopterus									X		X				X	
Pteronotropis signipinnis									X		X				X	
Pteronotropis welaka					X		X		X		X					
Rhinichthys atratulus			X		X								X			
Semotilus atromaculatus	X		X		X		X		X		X		X		X	
Semotilus thoreauianus	X		X		X		X		X		X		X		X	
total species	22	8	23	9	23	10	21	6	24	5	26	8	25	7	21	4

Chapter 2: Predicting Biological Interactions: Morphology as a Risk Assessment for Introduced Species

Abstract

Risk assessments are used to predict if introduced species can establish viable populations within its recipient community and can potentially become invasive. Many of these studies focus on the fundamental niche of the introduced species but lack questions about the realized niche of the species in its recipient ecosystem, despite the known importance of community effects within the recipient communities. Native species can either compete with introduced species or exclude them from the community, themselves be extirpated from the community, or not compete at all with introduced species. Morphology can shed light on an species ecology, thus comparison of the morphologies of native and introduced species through morphological overlap may provide insight into potential competitive relationships between them. In my study, the most successful introduced cyprinid species in the Mobile Bay Basin had no morphological overlap with native species. However,introduced species that have not been successful in establishing long-term populations shower substantial overlap with native species.

This leads to the conclusion that morphological overlap can aid in predicting the success of introduced species, when combined with past risk assessments.

Introduction

Introduced species can potentially establish viable populations within a recipient ecosystem, and once established, such species can become invasive. Risk assessments are used to gauge the likelihood of an introduced species becoming invasive for a given ecosystem upon introduction (Kumschick and Richardson, 2013). Many of these assessments focus on the abiotic interactions and physical limitations of species, but most include only basic biological and ecological information, despite the fact that some studies indicate that life history and ecological traits can influence invasibility (Rosecchi et al., 2001; Kolar and Lodge, 2002; Simberloff, 2003; Marchetti et al., 2004;). Often, when risk assessments do include information on biological interactions among organisms, minimal research has been conducted to address such questions, or they include sparse information about invasibility elsewhere or species physical tolerances (Bomford, 2008). No broadly applied risk assessments have examined ecological effects from communities in which a new species is introduced, despite some communities being shown to resist invasion through community interactions (Kirichenko et al., 2013). In this context, morphology could be used to predict potential biological interactions, such as competition with native species by introduced species, and thus invasibility, within an ecosystem.

Ecomorphology (sensuKarr and James (1975)), is the study of variations in morphology among individuals, populations, species, and other communities, and how it relates toecological conditons (Leisler and Winkler, 1985). Competition and other biotic interactions often select for different body forms and life histories (Lack, 1947), whereas convergence selects for similar phenotypes (Losos, 1992). Thus, by examining overall body form,it is possible to predict the potential for competition, and hypothesize the likely success of introduced species across many taxa. In this context, introduced species with no morphological overlap would be less likely to compete with native species and to form a viable population, than would species with substantial overlap. A common way to determine if there is morphological overlap among species is to perform multivariate analyses of gross anatomical measurements (traditional morphometrics) or of landmark data (geometric morphometrics). Landmark data analyzed with geometric morphometrics retains the shape of the organism throughout the analyses, thus this method is more appropriatefor establishing shape differences among individuals and speceis (Zelditch et al, 2012).

Ecomorphological studies have been applied to many varieties of vertebrate taxa including bats (reviewed by Swartz et al, 2003)birds (reviewed by Leisler and Winkler, 1985), rodents (Bowers and Brown, 1982), various mammalian carnivores (Friscia et al., 2007), reptiles (Goodman, 2009), ungulates (Curran, 2013), cervids (Curran, 2014), aquatic birds (Ibanez, 2012) and ray-finned fish (Actinopterigii, Motta et al., 1995). A geometric morphometrics approach was used in many of the more recent studies listed, and they were able to link morphology to differences in diet and habitat use. With ecomorphological knowledge from many taxa, the use of morphology can be applied to several taxa to discover potential the competitors of introduced species in an ecosystem.

Extensive functional morphological research has been conducted on the ecomorphology of ray-finned fishes. Mouth position is a strong predictor of feeding behavior. The position of the mouth reflects where in the water column the species feeds. Fishes with inferior mouths feed in the benthos, terminal mouthed fishes tend to be generalists, and fish with superior mouths feed at the surface, or pleuston (Alfaro, 1991). Relative mouth size often reveals the trophic position of a fish species: fish with a larger relative mouth size tend to be predaceous, species with a smaller relative mouth size tend to be herbivorous or detritivorous, and omnivores have an intermediate mouth size (Antonucci et al., 2009). The preferred flow regime occupied by fishes is also evident in their morphology. More fusiform, shallow-bodied fish prefer lotic (flowing-water) habitat, whereas deep-bodied fish prefer lentic (standing or low-flow) habitats (Cureton and Broughton, 2014). Position of the pectoral fin also affects the mobility and agility of fish species and thus can influence habitat use. High aspect ratio pectoral fins allow for greater swimming speed, and are typically found in fishes in the open water column (Wainwright et al., 2002). In contrast, fish with low pectoral fin aspect ratios tend to spend more time in structurally complex habitat and are slow swimmers, but have greater maneuverability than fish with high aspect ratios (Wainwright et al., 2002). Higher pectoral fin position on the body often indicates a burst swimmer, whereas endurance swimmers typically possess a lower pectoral fin (Costa and Cautadella, 2006). This extensive amount of information makes ray-finned fishes good candidates for morphometric studies.

By examining the overall shape of fishes through geometric morphometrics, one can examine many critical ecomorphological characteristics simultaneously. Principal components analysis (PCA) provides an effective summary of this multivariate information and allows one to potentially examine the extent of resource use overlap.

Many of the invasive aquatic fishes throughout the eastern United States belong to the family Cyprinidae (minnows, carps, shiners, and chubs). The Mobile Bay Basin contains a total of 60 cyprinid species, 6 of which are introduced (Boschung and Mayden, 2004), including the Fathead Minnow (*Pimephales promelas*), Red Shiner (*Cyprinella lutrensis*), Goldfish

(*Carassius auratus*), Common Carp (*Cyprinus carpio*), Silver Carp (*Hypophthalmichthys molitrix*), and Bighead Carp (*Hypophthalmichthys nobilis*). The Fathead Minnow (Smith-Vaniz, 1968) and Red Shiner (Kimsey and Fisk, 1964) have been continuously introduced for decades to this basin by anglers as baitfish from their home ranges west of the Mississippi River. Bighead and Silver Carps from Asia often are introduced to control phytoplankton (Robison and Buchanan, 1988), are accidentally released from aquaculture (Freeze and Henderson, 1982), or accidentally released due to stock contamination of Common Carp (Middlemas, 1994). Goldfish, also from Asia, often are released by pet owners, escape from hatcheries (Pflieger, 1997), used as baitfish (Litvak and Mandrak, 1993), and were widely introduced throughout the U.S. as ornamental fish in the 1800's (Dekay, 1842). The Common Carp, from Asia and Europe, is widely introduced as a sport fish and food fish (Litvak and Mandrak, 1993). Even with multiple introductions, the Red Shiner and Fathead Minnow have failed to establish permanent populations in the basin except in highly degraded areas (Boschung and Mayden, 2004).

Given these various cyprinid introductions, combined with extensive pre-existing knowledge of ray-finned fish ecomorphology, introduced cyprinids in the Mobile Bay Basin make a suitable taxon to test whether introduced and established species differ in their morphology, and thus predict competitive interactions between the native and introduced species. The objective of this study is to investigate if morphological traits can be used as a predictive tool to determine if introduced species will be successful in a recipient ecosystem.

Methods

Photographs of specimens of all native and introduced species of Cyprinidae in the Mobile Bay Basin were taken from specimens cataloged in the Auburn University Museum of

Natural History (AUMNH) Fish Collection. Native specimens were selected across collections that covered their home ranges in the basin, to maximize intraspecific variation for eachspecies. From the collections, the best specimens were selected, categorized by straight bodies that were adult sized except for the Asian carps, which have much larger adult sizes than the native species and can reach 120 cm in length. Smaller specimens of the Asian carps were examined, commensurate with native adult sizes, because they are more likely to compete with the native cyprinids at these sizes. Photographs were taken of 25 specimens in each species except *Carrassius auratus* (6 specimens), *Cyprinus carpio* (13), and *Hypophthalmichthys molitrix* (12), due to limited collection material. Specimens were photographed in lateral view with a Nikon D90 digital SLR camera attached to a copy stand. A file was created with the TpsUtil 1.46 program (Rohlf, 2010) and landmarks from Armbruster (2012) were digitized in TpsDig 2.16 (Rohlf, 2010). The tps file was converted to an NTSPC file in TpsUtil and then analyzed in MorphoJ (Klingenberg, 2011). Landmarks were aligned with a Generalized Procrustes Analysis (GPA). The data were checked for outliers which were removed (only 4 data points were removed), a covariate matrix constructed, and Principal Components Analyses (PCA) were performed. Data for each species generated by the landmarked photographs were then condensed into a single consensus point (one data point in the PCA per species). The GPA, PCA, and consensus points were obtained using MorphoJ (Klingenberg, 2011).

Three separate analyses were performed: one for only the native species of the Mobile Bay Basin, the second for the native species and introduced species, and a third for the genus

Cyprinella in order to test for morphological overlap. Cyprinella was examined, because there is one introduced, unsuccessful species and 6 native species in the Mobile Bay Basin. To examine how the invasive species influenced shape space overall, average nearest neighbor distance (NND) was performed on the Mobile Bay natives dataset, and on the native and introduced species dataset. The average NNDs were then compared between only the native species, and in the analysis that combined native and introduced species. To examine morphological variation on the genus level, a separate morphospace using the same methods above was generated for only the introduced Red Shiner (*Cyprinella lutrensis*) and its congeners. The red shiner has been known to reproduce with some of its congeners in the basin (Devivo and Freeman, 1995), and has yet to persist long-term in the basin (Boschung and Mayden, 2004)

A distance map was generated for each dataset, to determine how introduced species influenced overall shape space of the species found in the basin, using Spatstat in R (R-development team, 2008). The program utilizes PCA scores to form distance maps, which display the distance from various pixels to the nearest data points generated via the PCA.

Results

Shape space was generated using PC1 and PC2 and together described 63.7% of the total variation of all native and introduced cyprinids. There was no overlap between the Eurasian and native species, whereas some morphological overlap occurred between native species and the North American red shiner and the fathead minnow (Fig. 1). However, the fathead minnow and red shiner occupied a less dense area of shape space. Addition of the introduced species also increases the size of the shape space when compared to the shape space of the natural community, by increasing the values for the first principal component (Fig. 1).

Averaged shapes of grouped species are represented by wireframes. The wireframes differed between the introduced species combined with the native species versus the native species alone. The introduced species have a much deeper body in comparison to native species of the Mobile Bay Basin. The wireframe of the Eurasian species is the deepest-bodied (Figure 2).

Average NND for the morphospace containing only native species was 0.00488. The average NND for all native and introduced North America species combined was 0.00541. The average NND for the morphospace with all native and introduced species was 0.00633.

In the distance maps, the pixels closest to the data points are centered in the graph for only native species (Figure 3-A). In the North American dataset, the pixels closest to the data points maintain their shape with the exception of a slight shift to the right, due to the addition of 2 introduced species (Figure 3-B). When the dataset containing all cyprinids (introduced and native species) was analyzed, the distance map showed a further shift away from the original circular shape in the native species dataset (Figure 3-C). The PCAs used to generate the distance maps show the same pattern, with introduced species being added to the periphery of shape space by extending the PC1 vector (Figure 4). A shift in the colors of the map indicates that novel phenotypes have been introduced into the Mobile Bay Watershed.

Genus Level Comparison *of Cyprinella*.

For the comparison at the genus level between introduced and native *Cyprinella* species, morphospace was generated fromPC1 and PC2which explained 49.87% of the totalvariation. The introduced *C. lutrensis* had almost no morphological overlap with native species, with only one specimen of *C. gibbsi* occupying *C. lutresnsis'* 95% confidence ellipses. Hybrid *C. lutrensis* x *C. venusta* did overlap both *C. lutrensis* and some other species of Cyprinella, but the hybrid was distinct from *C.* venusta (Figure 5).

Discussion

Given the knowledge of ray-finned fish ecomorphology, morphology can be a good indicator of potential competition between native and introduced species, if correct morphological characteristics are measured. In my study, the addition of introduced cyprinids increased overall morphospace, as indicated by the higher average NND distance in comparison with the morphospace comprised of native species alone. However, this pattern was only true when the Asian carp species were included. Increased NND indicates that novel phenotypes have been introduced into the Mobile Basin. When only native species and introduced North American species were included, average NND also increased, but by a lesser degree than when the Asian cyprinids were included. Introduced North American species (*Cyprinella lutrensis* and *Pimephales promelas*) have not been able to establish viable, lasting populations in the Mobile Bay Basin (Boschun and Mayden, 2004). While these species were added to the periphery of morphospace, they may not be distanced enough from the native species to allow for a competitor release.

Distance maps also showed that as introduced species were added, there was a shift in the morphospace, with the most drastic shift occurring with the addition of Asian cyprinids. Like with NND results, this shift suggests that new morphologies were added to the basin. Moreover, the wireframe of the average shape of the native species was much shallower in body than that of average cyprinid shape of all of cyprinids, including introduced species. The wireframe of only Asian species (Figure 2C) was the deepest bodied of all the wireframes, thus extending PC1 along its axis and changing overall shape of distance maps (Figure 4). Introduced species appear to be changing the average cyprinid shape towards a larger head and deeper body. This result indicates that habitat preferences of the introduced species may differ from those of the native species, in that the introduced species may prefer more lentic habitats.

Hypophthalmichthys showed no morphological overlap with native cyprinids. This lack of overlap may reflect a difference in habitat use with native species, since PC1 showed a difference in body depth. Body depth within fishes correlates to habitat use, with deeper-bodied fish preferring slow water movement (Cureton and Broughton, 2014). Both species of *Hypophthalmichthys* prefer lentic habitats, whereas most native cyprinids prefer lotic habitats (from species accounts in Boschung and Mayden, 2004).

Cyprinus carpio, the common carp, is another species of Asian carp that showed no morphological overlap with native species. The common carp is a lentic, omnivorous species that feeds primarily on insects, annelids and macrophytes (Boschung and Mayden, 2004). Its diet does not overlap with the majority of native cyprinid species, and neither does its primary habitat use. This means that the common carp could experience a competitive release in the Mobile Bay Basinin relation to other members of its family.

The Red Shiner is a widely-introduced species throughout the United States, and was introduced to the Mobile Bay Basin incidentally as a baitfish (Kimsey and Fisk, 1964). The red shiner has established populations only in degraded habitats throughout the Mobile Basin (Boschung and Mayden, 2004), likely due to its high tolerance of a varied range of environmental conditions. This species can thrive in low flow, low oxygen, acidic, and/or otherwise polluted waterways (Matthews and Hill, 1977, 1979; DeVivo and Freeman, 1995;

Matthews, 1985). Thus, as urbanization in the Mobile Basin increases, the Red Shiner will likely becomes more of a threat to invade its river systems (Devivo and Freeman, 1998). It also morphologically overlaps with a few of the deeper-bodied native species, such as the Golden Shiner that do not persist in these degraded habitats. The Red Shiner could experience competitive release in the degraded areas, but is unable to out-compete the native species in less-disturbed areas. However, when introduced to more pristine areas, it freely hybridizes with many of its congeners (Walters et al., 2008). The Red Shiner's hybrid with *Cyprinella venusta* overlaps some of its congeners in the Mobile Bay Basin. Hybridization not only dilutes the gene pool of native species, but the hybrids may be competing with native species because ofmorphological overlap with congeners. This species is of particular interest, given its ability to invade; a recent study suggests that the Mobile Basin is of particular susceptibility to Red Shiner invasion under current and future climatological conditions (Poulos et al., 2012).

The fathead minnow is another commonly-used baitfishintroduced to the Mobile Bay Basin multiple times (Smith-Vaniz, 1968). Like the red shiner it has been unsuccessful in establishing populations in natural habitats (Boschung and Mayden, 2004), but has established populations throughout the United States in impoundments and muddy stream pools. The Fathead Minnow can survive in a wide range of environmental conditions, which makes it a suitable invasive species (Held and Peterka, 1974; Klinger at al., 1982). Its abilities to survive in anoxic water and to subsist on detritus when macroinvertebrates are scarce both allow it to persist in degraded habitats (Herwig and Zimmer, 2007). It is also very fecund and can reproduce in its first year, allowing this speceis to quickly become a dominant species in the habitats it invades (Gale and Buynak, 1982). The reason why this speceis has failed to establish in the Mobile Bay Basin is unknown, but my results suggests that competition could play a factor in this prevention, due to its close proximity to native fish in morphological shape (Figure 1).

In my study, all invasive species showed deeper bodies and enlarged heads than the native species. Such physical attributes could serve as indicators for potentially invasive cyprinid species in the Mobile Bay Basin in the future. This method can help provide morphological indicators for potentially invasive species, and sound management decisions can be implemented before the potentially invasive species are introduced into a new environment. For example,cyprinid species with deeper bodies and larger heads in relation to body size could be blacklisted for import, thus limiting if not completely eliminating their effects on new ecosystems (Reichard and Hamilton, 1997).

Using morphology to predict interactions between native and introduced species has its limitations. While this approach can be used as a tool to predict competition or possible competitive release for introduced species, it cannot be used to predict other important interactions among species such as predation. It would, however, indicate whether a predatory niche would be available to an introduced predator. Predator-prey relationships for fish can also be surmised through traditional morphology by measuring the gape of the newly introduced predators and the overall body size of pre-existing prey species. If prey species can be easily engulfed by a predator, then they are more likely to be among the group upon which the predator preys (Schael et al., 1991). Using geometric morphometrics may not always be the best tool to predict other interactions beyond possible competition.

Although this study is a retrospective survey including only known successful or moderately successful exotic species, it demonstrates that geometric morphometrics can describe habitat preference and identify potential competition between native and introduced species, as well as traditional morphometrics to infer which species can be engulfed by introduced predators, predator-prey interactions can be inferred. With the addition of the abiotic factors which traditionally are taken into account in most risk assessments, conservation and invasion scientists can gain valuable information for predicting which species may become successful and thus invasive if introduced, and can then make ecologically-sound management decisions designed to protect native species. The method outlined here is relativelyrapid and inexpensive, and morphology can be used as a proxy for understanding the ecological distinctiveness of exotic species.

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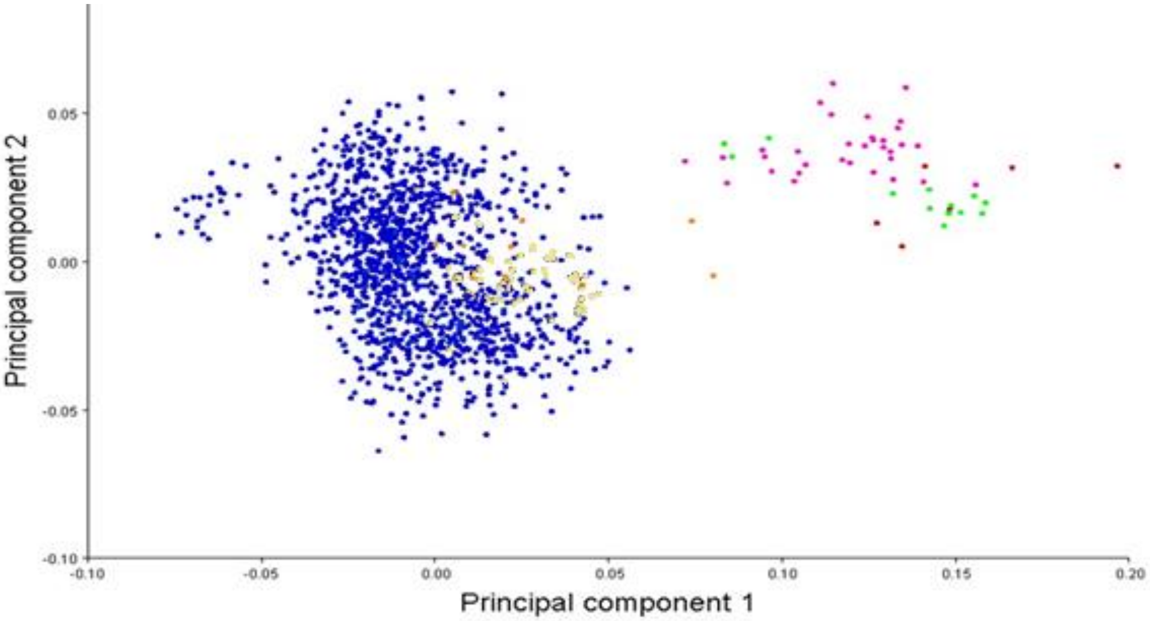

Figure 1: Shape space for each of – [how many?] cyprinid species in the Mobile Bay Basin. The blue points are native species. The other 5 colors of points are for introduced species: tan are *Cyprinella lutrensis*, orange are *Pimephales promelas*, pink are *Hypophthalmichthys* species, red are *Carassius auratus*, and green are *Cyprinus carpio*. PC1 and PC2 described 63.2% of the data.

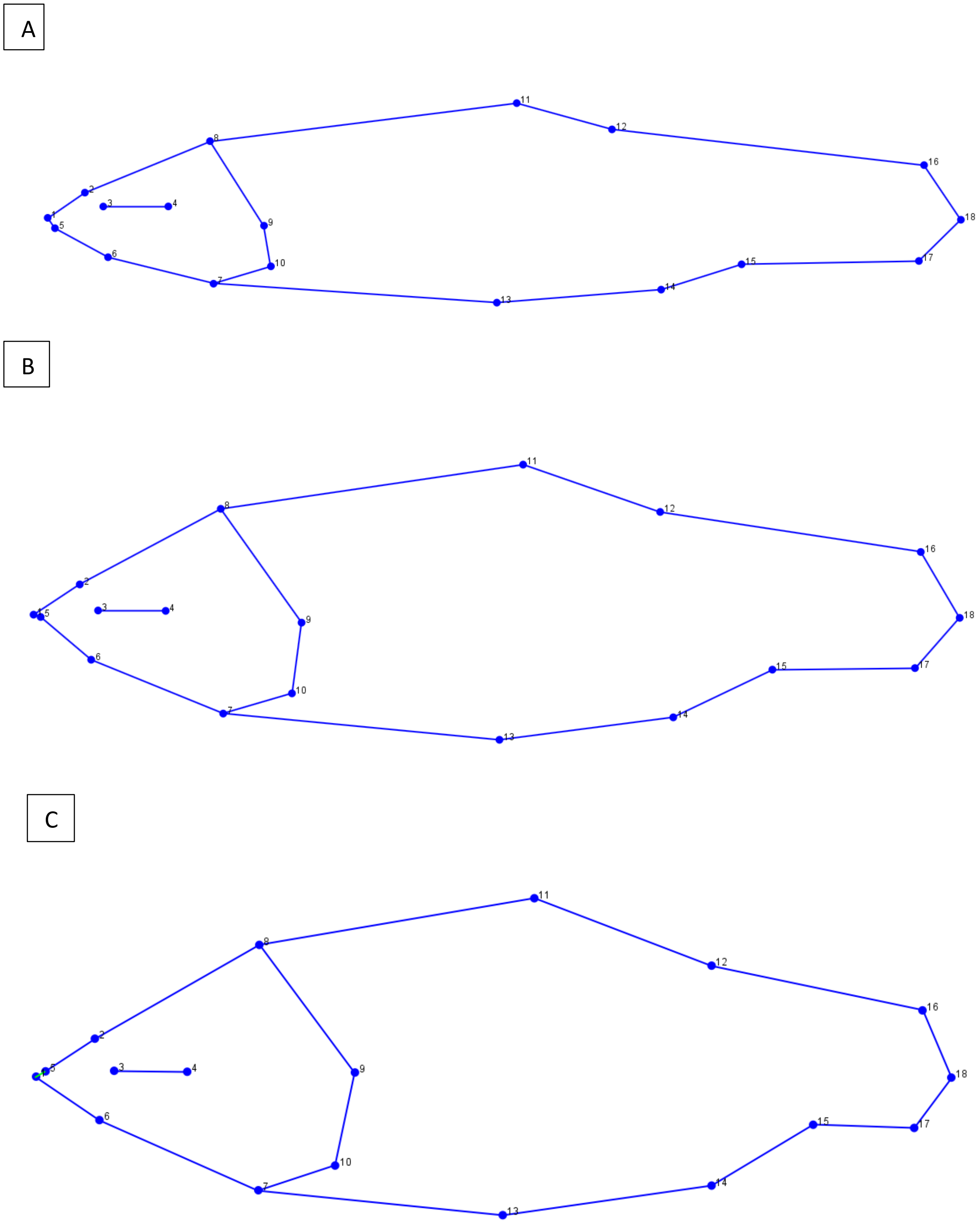

Figure 2: Wireframes, the average shape of fishes derived from landmark data. A) represents the wireframe of the native species. B) represents the wireframe of the introduced species. C) represents the wireframe of the introduced Asian species.

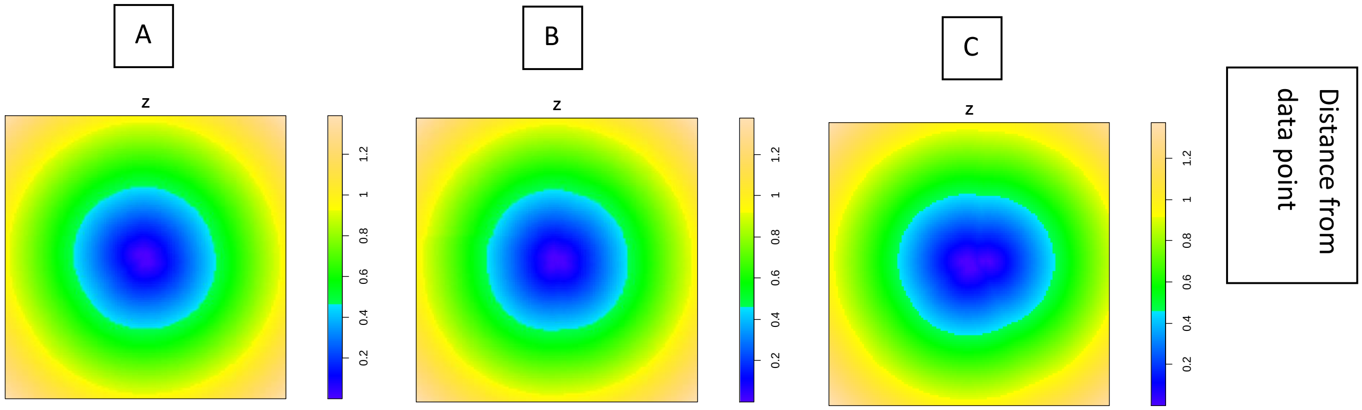

Figure 3. Distance maps for (A) only natives dataset (B) North American dataset (C) All cyprinids dataset. The cooler colors in the distance maps indicate that the pixels in the image are closer to data points whereas warmer areas indicate greater distance from that pixels to a data point.

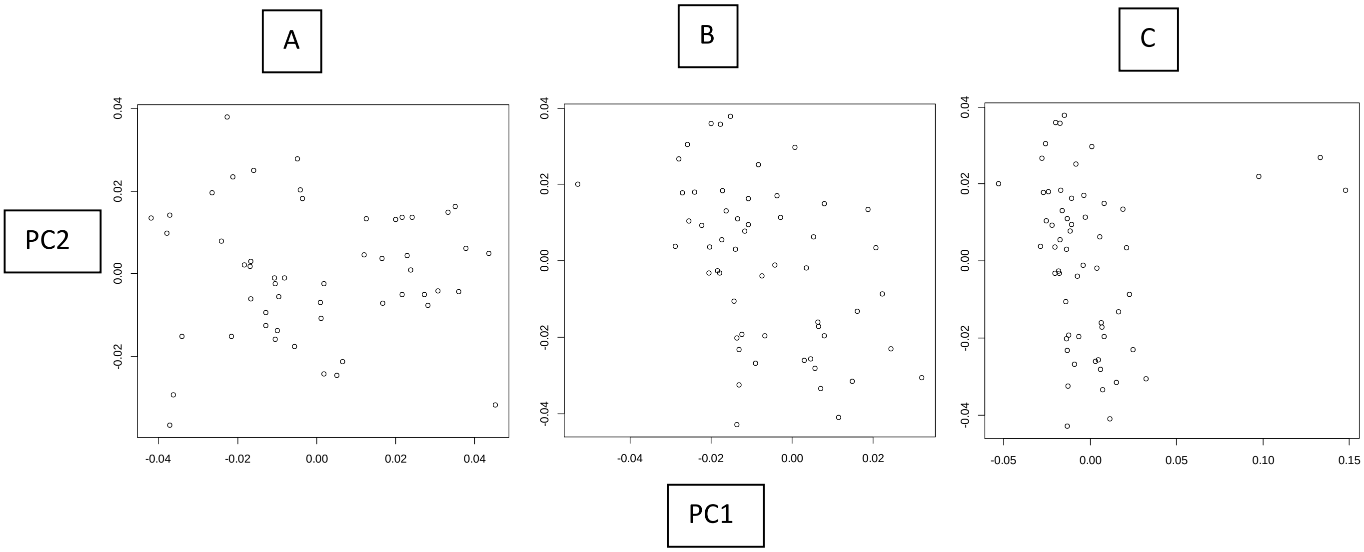

Figure 4. PCA for (A) only native species (B) only North American species (C) all cyprinids.

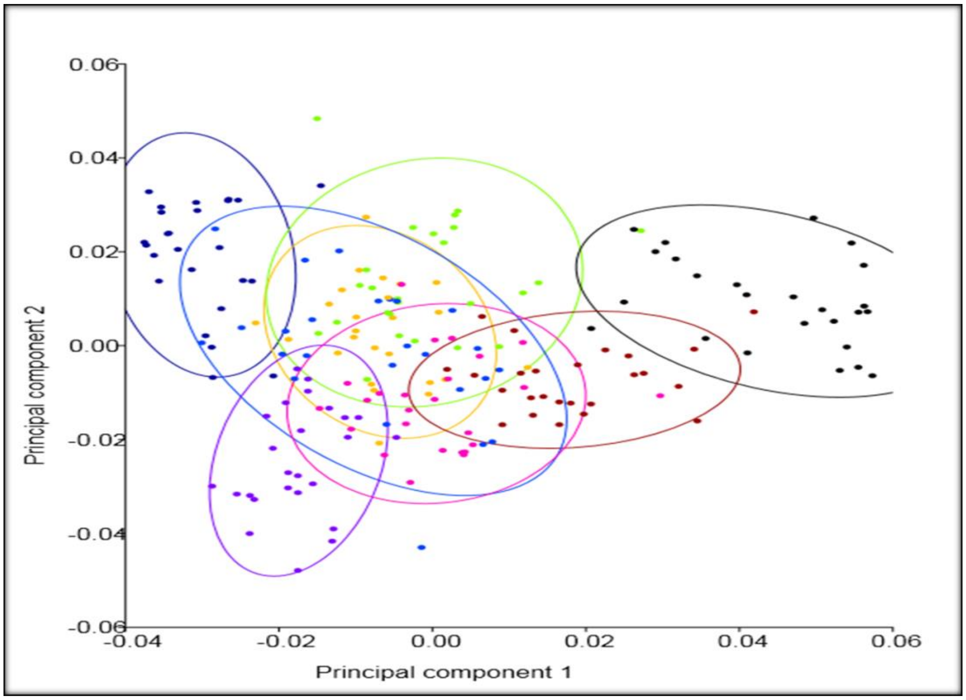

Figure 5. PCA for the genus *Cypinella* with 95% confidence intervals. PC1 described 30.7% of the data. PC2 described 19.2%. Black data points are *Cyprinella lutrensis* specimens, blue points are *Cyprinella caerulea*, purple points are *Cyprinella venusta*, green points are *Cyrinella gibbsii*, blue points are *Cyprinella trichroistia*, orange points are *Cyprinella callistia*, pink points are *Cyprinella whipplei*, and red points are the *C. lutrensis* and *C. venusta* hybrids.

Appendix A

Specimens used for study.

Species	Catalog Number	# specimens
Campostoma oligolepis	AUM 50134	8
	AUM 57068	17
Carassius auratus	AUM 2976	1
	AUM 5537	1
	AUM 15221	2
	AUM 32164	1
Cyprinus carpio	AUM 5174	10
	AUM 10374	3
Cyprinella caerulea	AUM 25208	5
	AUM 62253	20
Cyprinella callistia	AUM 40371	11
	AUM 41197	4
	AUM 46907	10
Cyprinella gobbsi	AUM 50219	11
	AUM 56868	14
Cyprinella lutrensis	AUM 39644	19
	AUM 57669	3
	AUM 58470	3
C. lutrensis x *C. venusta*	AUM 26612	22
	AUM 33640	3
Cyprinella trichroistia	AUM 49370	10
	AUM 49386	7

	AUM 51787	8
Cyprinella venusta	AUM 34949	15
	AUM 35084	10
Cyprinella whipplei	AUM 13854	5
	AUM 17679	20
Hemitremia flammea	AUM 12708	10
	AUM 12711	3
	AUM 12713	12
Hybognathus hayi	AUM 895	2
	AUM 5538	7
	AUM 19535	6
Hybognathus nuchalis	AUM 56894	25
Hybognathus winchelli	AUM 31028	25
Hybopsis aestivalis	AUM 23092	22
	AUM 18363	3
Hybopsis lineapunctata	AUM 6830	7
	AUM 42379	13
	AUM 56959	5
Hypophthamichthys molitrix	AUM 12193	4
	AUM 12209	1
	AUM 23020	7
Hypophthamichthys nobilis	AUM 24733	25
Luxilus chrysocephalus	AUM 57066	19
	AUM 58401	6
Luxilus zonistius	AUM 33252	15

	AUM 57312	10
Lythrurs atrapiculus	AUM 10754	2
	AUM 2130	4
	AUM 3766	5
	AUM 10860	14
Lythrurs roseipinnis	AUM 53323	9
	AUM 54665	13
	AUM 56377	3
Lythrurus alegnotus	AUM 48858	6
	AUM 27058	19
Lythrurus bellus	AUM 42709	13
	AUM 55184	12
Lythrurs fasciolaris	AUM 3190	25
Lythrurus lirus	AUM 3126	24
	AUM 25266	1
Macrhybopsis storeiana	AUM 29007	15
	AUM 39971	10
Nocomis leptocephalus	AUM 31187	25
Notemigonous crysoleucas	AUM 336	3
	AUM 6686	5
	AUM 7060	17
Notropis volucellus	AUM 6180	25
Notropis ammophilus	AUM 42457	25
Notropis amplamala	AUM 49899	25
Notropis asperifronss	AUM 19286	4

	AUM 57975	21
Notropis atherinoides	AUM 39753	13
	AUM 54908	12
Notropis baileyi	AUM 42401	25
Notropis cahabae	AUM 5685	12
	AUM 7979	6
	AUM 20891	4
	AUM 55463	3
Notropis candidus	ACN21901	13
	AUM 13653	1
	AUM 15493	3
	AUM 15643	3
	AUM 20301	5
Notropis chalybaeus	AUM 8917	10
	AUM 9002	10
	AUM 28882	5
Notropis chrosomous	AUM 40493	11
	AUM 58402	14
Notropis edwardraneyi	AUM 9227	1
	AUM 20673	5
	AUM 21817	6
	AUM 21830	12
	AUM 56898	1
Notropis longirostris	AUM 758	25
Notropis maculatus	AUM 6688	25

Notropis petersoni	AUM 31078	25
Notropis stilbius	AUM 49301	25
Notropis texanus	AUM 14763	25
Notropis uranoscopus	AUM 6178	12
	AUM 18367	13
Notropis xaenocephalus	AUM 31820	15
	AUM 32090	10
Opsopoedus emiliae	ACN866	12
	AUM 7869	1
	AUM 26368	12
Phenacobious catostomus	ACN1198	25
Pimephales promelas	ACN1156	1
	ACN2469	2
	AUM 22920	1
	AUM 29748	2
	AUM 5546	1
	AUM 52923	3
	ACN8237	1
	AUM 8531	1
	AUM 13116	1
	AUM 20989	1
Pimphales vigilax	AUM 39646	4
	AUM 57241	11
	AUM 59086	10
Pimephales notatus	AUM 52900	25

Pteranotropis hypselopterus	AUM 25991	25
Pteranotropis signipinnis	AUM 6256	25
Pteranotropis welaka	AUM 21214	4
	AUM 26746	1
	AUM 28860	5
	AUM 42083	15
Rhinichthys atratulus	AUM 56254	25
Semotilus atromaculatus	AUM 53066	6
	AUM 52978	10
	AUM 25914	9
Semotilus thoreauianus	AUM 25911	7
	AUM 25916	18