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A Change in Fish Assemblages in the Pascagoula River, MS

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A CHANGE IN FISH ASSEMBLAGES IN THE PASCAGOULA RIVER, MS

by

Sara Barrett

A Thesis
Submitted to the Graduate School,
the College of Arts and Sciences
and the School of Biological, Environmental, and Earth Sciences
at The University of Southern Mississippi
in Partial Fulfillment of the Requirements
for the Degree of Master of Science

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ABSTRACT

The Pascagoula River in southeastern Mississippi is the largest remaining unimpounded river system in the contiguous United States and have seen a change in fish assemblages over time. Examination of long-term fish assemblage data show that the mainstem Pascagoula River and its two major tributaries (mainstem Leaf and Chickasawhay Rivers) have been dominated by five abundant taxa that comprise 83% of individuals sampled, with *Cyprinella venusta* in the highest abundance. Beginning in about 2005-2006, a change in the mainstem Pascagoula River (but not mainstem Leaf and Chickasawhay rivers) was seen to an alternate assemblage where five different taxa dominate (89% of assemblage), with *Hybognathus nuchalis* now the most abundant species. Thus, a shift in fish assemblages seems to have occurred in one portion of the drainage but not the other. A potential driver for the switch in historic versus contemporary assemblage composition could have been changes in the abiotic environment, most specifically in dissolved oxygen (DO) within the Pascagoula River. Because of the differences in tolerances to the abiotic environment, *H. nuchalis* may be able to utilize a broader variety of habitats compared to *C. venusta* and be a notable factor in the reason there is a shift in relative abundances in assemblages. *Hybognathus nuchalis* can withstand pools and backwaters that are lower in DO and might do better in more extreme conditions, while *C. venusta* is unable to tolerate the harsher environments.

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LIST OF ABBREVIATIONS

<i>USM</i>	The University of Southern Mississippi
<i>MMNS</i>	Mississippi Museum of Natural Science
<i>ANOVA</i>	Analysis of Variance
<i>NMDS</i>	Non-metric Multidimensional Scaling
<i>PERMANOVA</i>	Non-parametric Multivariate Analysis of Variance
<i>CV</i>	Coefficient of Variation
<i>LEC</i>	“Loose” Equilibrium Concept
<i>DO</i>	Dissolved Oxygen
<i>BOD</i>	Biological Oxygen Demand
<i>mm</i>	millimeters
<i>ASR</i>	Aerial Surface Respiration
<i>LC50</i>	Lethal Concentration where 50% mortality is observed
<i>TDS</i>	Total Dissolved Solids
<i>N₂</i>	Nitrogen Gas
<i>ANCOVA</i>	Analysis of Covariance

CHAPTER I – A CHANGE IN FISH ASSEMBLAGES WITHIN THE PASCAGOULA RIVER AND THE POTENTIAL FOR AN ALTERNATIVE STABLE STATE

1.1 Introduction

Par A central research question in ecology focuses on assemblage dynamics and how assemblages change over time. The interest of this idea stemmed from the debate of early plant ecologists Frederic Clements and Henry Gleason. Clements considered assemblages to be a complex superorganism that followed a pattern of succession, which resulted in a stable climax assemblage. Clements' theory suggests predictable species turnover as the system proceeds through succession with characteristic species composition emerging in different points in time (successional stages). He believed that the successional direction of species after a disturbance is predictable, with it more or less returning to that same state (Clements, 1916 and 1936). Conversely, Gleason viewed assemblages as loose collections of species or individuals. In Gleason's individualistic idea, he notes that species can exist in different abundances and those abundances are affected by biotic interactions and physiological tolerances. Some species may succeed, while others may not be as successful post-disturbance. Gleason viewed species distribution as dependent on abilities of migration and environmental requirements, which can be altered after disturbances (Gleason, 1926). Others have also aimed at determining what drives assemblage dynamics and assemblage change over time. Studies of metacommunities aim to explain variation in assemblage composition using environmental and spatial variables (Heino et al., 2015) while others have used paleontological evidence (DiMichele et al., 2004) to address these questions. Assemblages can be subjected to fluctuations driven by density-independent, abiotic conditions as well as density-dependent, intra- and interspecific interactions (Gilpin

1979; May 1977). More recently, Liataud et al., (2019) recognizes the ideas of Clements and Gleason are just two limiting cases along a gradient of possible outcomes explaining assemblage composition. Assemblages are driven by biotic and abiotic processes that interact to form the dynamic system. However, whether or not assemblages have one single stable equilibrium point or not and what processes control equilibrium dynamics remain pertinent questions in ecology. It is important to understand all of the potential abiotic and biotic interactions in order to predict the response of species and assemblages to environmental changes, such as disturbances. In the case of disturbances, a drastic change in the environment could lead to abrupt shifts from one assemblage to another; however, this may not always be the case.

Assemblages in equilibrium may change after experiencing a disturbance but should eventually return to that same equilibrium point. Ecological theory suggests some assemblages may have multiple stable equilibrium points (hereafter stable states), with the potential for switching among them (Bertness et al., 2002; Sutherland, 1974, 1990; Peterson, 1984; Hollings, 1973). Stable states must persist long enough to establish they are not simply part of a successional or directional return to the one stable equilibrium point. Stable states are therefore defined on generation time (states persist over multiple generations of resident species) (Bertness et al., 2002, Connell and Sousa, 1983 and Peterson, 1984). Stable states can be quantified by abundances of species or guilds, or measurement of ecosystem functions (Pace et al. 1999). In this context, a stable state occurs when ecosystem biomass (Beisner et al., 2003) or patterns of individual species abundances do not change in the face of some disturbance or environmental variability (Ives and Carpenter, 2007).

Assemblages can shift between stable states into alternative stable states through various mechanisms (Augustine et al., 1998, Bertness et al., 2002, Scheffer et al., 2001 and Knowlton 1992). Both biotic and abiotic disturbances have been documented to facilitate stable state shifts. Biotic disturbances shown to drive stable state shifts include competition, predation (Paine 1966 and Bruno et al 2009), and facilitative reproduction (Baskett and Salomon 2010). In Pacific kelp assemblages, the abundance of urchin mediates switches between alternative stable states: kelp forest and an urchin barren state. A healthy kelp forest is home to a variety of marine species such as rockfish, leopard sharks, and great whales. The forest provides nursery habitat for marine species as well (Graham, 2004; Wilmers et al., 2012). Top predators (sea otters) regulate urchin populations and decrease grazing pressure by urchins. A decline in sea otter populations releases predation pressure on urchins, leading to heavy kelp grazing that decimates kelp populations. The urchin barren assemblages are dominated by crustose coralline algae and are considered stable because it provides a system that can survive generations of a consistent assemblage made up of herbivorous sea urchins and coralline red algae inhabitants. While it lacks in food web complexity and lower levels of productivity compared to the kelp assemblage, the urchin barren system is deemed an alternative stable state due the ability to maintain these assemblages over a generational scale (Filbee-Dexter and Scheibling, 2014).

State shifts may also be driven by abiotic disturbances (natural and anthropogenic). Turbidity changes and subsequent loss of submerged aquatic vegetation causes state shifts in shallow lakes (Carpenter et al., 2003, Scheffer et al., 1993, Jeppesen et al., 1999). Agricultural run-off and other anthropogenic factors increased turbidity in once clear lakes,

resulting in the loss of submerged aquatic vegetation. Turbid state shifts are undesirable because of the disappearance of submerged aquatic vegetation that leads to reduced fish diversity and biomass and high algal biomass. Through experimental work, it was shown that plants increase the water clarity which allows the once turbid lakes to become clear again (Scheffer et al., 1993; Capon et al., 2015). The mechanism for the switch from turbid state to clear lake state involves reduction of nutrients in the water column, protection of phytoplankton grazers against predation from fish, and prevention of sediment resuspension (Scheffer et al., 1994). Similar to what is seen in the kelp alternative states, the turbid lake state is recognized as a stable even though there is a loss of food web complexity and biodiversity. In both cases, some external force (biotic or abiotic) would be needed to change the states back to the previous state.

Not all disturbances can cause an assemblage to change states. Perturbations may alter assemblages briefly but return back to equilibrium (resilience) (Gunderson, 2000). Theories on alternative stable states suggest that states are separated by thresholds where an assemblage resides (e.g. its known stable state) until experiencing a perturbation large enough to push it outside of its threshold and into another state. If the disturbance is not severe enough, the assemblage will remain in equilibrium. Important properties of alternative stable states are resistance and resilience. Resistance is described as the ability for an ecosystem to remain unchanged after experiencing a disturbance or multiple disturbances. An assemblage can resist state shifts and tend to remain in one state unless the disturbances are large enough to push it outside of the threshold (Beisner et al., 2003, Schroder et al., 2005, Sutherland, 1974, Scheffer et al., 2001). Resilience is defined as the ability of a community to return to an equilibrium state after a disturbance (Gunderson,

2000). Assemblages can drastically shift from their state and still return to pre-disturbance conditions. However, some disturbances are strong enough to shift states out of their existing stable state and into an alternative state that does not return to pre-disturbance conditions (Folke et al., 2004). Both of these concepts are components of determining the fate of the system after a disturbance. While there is knowledge on alternative stable states, there is still much that remains to be understood about how these assemblage shifts could be impactful to the management and conservation of freshwater systems.

I have observed changes in fish abundances and believe that a potential stable state shift has been observed within the Pascagoula River system of southeastern Mississippi. The Pascagoula River System is a dynamic ecological system that has experienced natural and anthropogenic disturbances recently, including Hurricane Katrina in 2005, Deepwater Horizon Oil Spill in 2010, and a major drought in 2012 (NOAA, www.noaa.gov). The river is the largest remaining un-impounded river in the continental United States with a discharge of over 2.4 cu mi per year (Dynesius and Nilsson, 1994). The Pascagoula River is subjected to minor anthropogenic disturbances due to DeSoto National Forest protecting some of the surrounding land. Therefore, the hydrology of the Pascagoula is fairly pristine, making the river a good system to study naturally occurring alternative stable states. While there is fairly extensive literature on freshwater fish assemblage dynamics (Matthews and Marsh-Matthews, 2017; ST Ross, 2013), there have been few documented examples of alternative stable states in freshwater fish assemblages in the literature. Thus, there is not a lot of information on how changing stable states can occur and how this might influence species within the system.

The Pascagoula River basin is home to over twenty threatened or endangered terrestrial and aquatic species, including the Gulf Sturgeon (*Acipenser oxyrhynchus desotoi*) (USFWS, 1991) and the Pearl Darter (*Percina aurora*) (USFWS, 2017). The Pearl Darter was endemic to the Pascagoula and Pearl drainages, but it has been extirpated from the Pearl drainage after flood control and navigation structures were installed (Tipton et al., 2004). The species has recently been federally listed and is now only found in the Pascagoula River drainage (Suttkus et al., 1994, Schaefer and Mickle, 2011, Clark et al., 2018). Thus, knowledge on changes to fish assemblages within these rivers may have broader conservation implications for these listed species.

The Pascagoula River is formed at the confluence of the Chickasawhay and Leaf rivers. Interestingly, the fish assemblages in these two major tributaries have not appeared to experience shifts during this same period, meaning an alternative stable state shift may have occurred just in the Pascagoula River. Historical collection data of the Pascagoula River show that the potential stable state shift occurred sometime between 2005 and 2006. The changing fish assemblages of the Pascagoula River can be seen in some of the numerically dominant small-bodied cyprinids (*Cyprinella venusta*, *Hybognathus nuchalis*, *Notropis longirostris* and *Notropis atherinoides*). All four species have been recorded throughout all sampling years, but abundances have changed temporally. *C. venusta* made up about 61.1% of samples before 2005, compared to only 8.5% of samples after 2006. In that same period, *H. nuchalis* only made up 2.4% of samples before 2005, and now makes up 68.5% of samples after 2006.

While changes in abundance were seen in the top five most abundant taxa, I was interested in the entire assemblage to see if a stable state shift occurred in the Pascagoula

River. My objectives of this study were to analyze the assemblage composition within the Pascagoula, Leaf, and Chickasawhay rivers to compare contemporary assemblages to historical assemblages. I also took a null model approach to determine if assemblage change in the Pascagoula River were part of normal population fluctuations that are expected through stochastic processes (Schaefer et al., 2005), or the result of a stable state shift. Specifically, I asked: 1. Has there been a change in fish assemblages in the Pascagoula River? 2. Is there statistically significant variation in assemblages over time in the three rivers? 3. Are fish assemblages stochastic enough to have changed into alternate stable states through random processes?

1.2 Methods

1.2.1 Two Long-term Datasets

Data used in this project range from 1976-2019 with some variation in sampling effort and gear used. Historical data was compiled from collections from University of Southern Mississippi (USM) Ichthyology Collection (Hattiesburg, MS) and the Mississippi Museum of Natural Science (MMNS) Ichthyology Collection (Jackson, MS). Contemporary (2009-2019) collections used consistent gear and effort and are cataloged in the USM Ichthyology Collection.

Because of variability in effort and gear (primarily boat electrofishing and seining), not all of the dataset was directly comparable. I therefore constructed two datasets used in different analyses. The first dataset (hereafter called Ratio Dataset), combined all available data (1976-2019) and only used the ratio of abundances between the two most abundant species that characterized the observed changes (*C. venusta* and *H. nuchalis*). The second dataset contained samples from 2000-2019 (hereafter called Assemblage Dataset) where

the sampling effort and gear (details described below) were standardized. For both datasets, I refer to samples before 2006 as historic and all samples since as contemporary.

1.2.2 Ratio Dataset

1.2.2.1 Fish sampling

Seines varied in sizes and were between 4.6-9.1 m in length, typically 1.2 m deep, and either 3.2 or 4.8 mm mesh. Seining effort (n = 733) was typically 30-45 minutes per site and generally took place along sand bars, but backwaters and other available habitats were also sampled. Sampling occurred at various times of the year between April and November. For sampling done by boat electrofishing (n = 14), each site was electrofished for 400 seconds and all fish were identified on site then released (Mickle 2006; Schaefer et al., 2006). Sites contained sand bar, open channel, and bank habitats. Fish from seining were preserved in 10% formalin, identified in the laboratory, transferred to 70% ethanol, and then vouchered in the USM or MMNS Ichthyology Collections.

1.2.2.2 Analyses

The Ratio dataset was used to describe the relative proportions of *C. venusta* and *H. nuchalis* to distinguish between different community types within the rivers. The final dataset contained 747 collections over 40 years (1976-2019) with 316, 229, and 202 samples in the Chickasawhay, Leaf, and Pascagoula rivers respectively (Figure 1). To generate the proportions, I divided the total number of *H. nuchalis* in a sample by the sum of the number of *C. venusta* and *H. nuchalis* in a sample. I then arcsine transformed our proportion data. I used a two-factor analysis of variance (ANOVA) to test for differences in the proportions of *H. nuchalis* to *C. venusta* in each of the three rivers between historic

and contemporary samples. Significant pairings were done using a TukeyHSD Post Hoc test.

1.2.3 Assemblage dataset

1.2.3.1 Fish sampling

In this dataset sampling efforts and gear were consistent throughout all sampled years including 2000-2019. There were 59 total sites that were sampled: 30 in the Pascagoula, 16 in the Chickasawhay, and 13 in the Leaf. With those 59 sites sampled over multiple years, there were 191 samples in the Chickasawhay, 171 samples in the Leaf, and 125 samples in the Pascagoula River (Table 1). Overall, there were 483 samples over 19 years, with individual count data for 105 species. Rivers were not sampled an equal number of times each year, but all sampling occurred between April and November. Sites were typically sand banks along the outer perimeters of the rivers. Fish were collected by 1.8×6.1 mm (3.1 mm mesh) double-lead seine. Seining generally took place in thirty-meter reaches and total sampling effort for the entire site was 30-45 minutes. Fish collected from sampling were fixed in 10% formalin immediately upon capture and taken back to the lab to identify and count. Fish were preserved in 70% ethanol and vouchered in the USM Ichthyology Collection (USM lot numbers 56458-57508).

1.2.3.2 Analyses

Data were analyzed to assess if assemblage patterns observed historic assemblages were different with what was sampled in the rivers in contemporary samples. Species that occurred less than three times or had an abundance of less than five, as well as samples that had fewer than three individuals or three species, were removed from the analyses. Rare species were removed because I am interested in the dynamics of the core species and rare

species may represent low capture probabilities and prevalence, thus are not as ecologically informative for our research questions.

To analyze whether fish assemblages have changed from what was sampled historically, I conducted a series of statistical tests to determine the amount of assemblage change in each of the three rivers. First, I used non-metric multidimensional scaling (NMDS) as a descriptive technique to observe assemblage patterns. For the NMDS, I used Bray-Curtis dissimilarity matrices (each river analyzed independently) to see whether assemblages are grouped temporally in ordination space or whether all samples grouped similarly. I subsequently ran a non-parametric Multivariate Analysis of Variance (PERMANOVA) to test for differences in assemblages between historic and current samples within each river.

Finally, I took a null model approach to determine whether the changes in fish assemblage composition could have happened by stochastic processes or the results of a stable state shift. The null model follows the guidelines of Schaefer et al. (2005) to create a model of expected community changes. It is based on the relationship between species abundance and the coefficient of variation (CV) in abundance of all historic assemblages. Historic assemblages were chosen because I am interested in determining if the contemporary changes are more different from the historic assemblages than what is expected by stochastic processes. I calculated the mean and coefficient of variation (CV) in abundance for each species. Then, I fit a model that predicted the expected CV in abundance over time for each species and generated random assemblages from the model. The model looks at each species independently, so no biotic interactions are accounted for in the model. Also, the model assumes each sample is independent of the previous sample

through time. I plotted the assemblages (random and observed) from the generated random assemblages in ordination space using NMDS. I built frequency distributions based on the Euclidean distances between each assemblage (random and observed) and the centroid for all historic species points in ordination space. The distributions were then used to test the hypothesis that the observed assemblages have changed less than would be expected at random due to natural fluctuations. Any observed contemporary samples that had a Euclidean distance from the centroid of the randomly generated assemblages of greater than 95% were deemed as significantly changed.

1.3 Results

1.3.1 Ratio dataset

Of the 747 samples within this dataset, 553 samples had a higher ratio of *C. venusta* than *H. nuchalis*. Within each river, 204 of these samples are in the Chickasawhay, 260 are in the Leaf, and 89 in the Pascagoula River. There were 91 samples where both *C. venusta* and *H. nuchalis* were absent from collection with 8 samples in the Chickasawhay, 58 in the Leaf, and 25 in the Pascagoula River.

The results of the ANOVA found that there was a significance effect of river and time period on the number of samples that had a higher proportion of *H. nuchalis* to *C. venusta* (River: $F_{1,2}= 305.6$, $p<0.0001$; Period: $F_{1,1}= 197.9$, $p<0.0001$; River \times Period: $F_{1,2}= 145.3$, $p<0.0001$) (Table 3). There was also a significant interaction indicating that there were differences between the two periods within each of the three rivers on the number of sites that had a higher proportion of *H. nuchalis* to *C. venusta*. A TukeyHSD Post Hoc test indicated that the Leaf and Chickasawhay Rivers were grouped together in the ANOVA (p

= 0.075), while the Pascagoula River is driving the significant differences in proportions of *H. nuchalis* to *C. venusta*.

1.3.2 Assemblage dataset

There were 59 sites sampled altogether within each of the three rivers. There were 30 sites in the Pascagoula, 13 in the Leaf, and 16 in the Chickasawhay rivers. All sampling took place within these sites throughout all years within the Assemblage dataset; however, not all 59 sites were sampled in each year. Analyses of historical collections of the Pascagoula River showed there were initially five species that were most abundant, (*Cyprinella venusta* (61.1%), *Trinectes maculatus* (5.6%), *Notropis longirostris* (4.6%), *Pimephales vigilax* (4.0%), and *Anchoa mitchilli* (3.0%)), comprising of about 83% of individuals sampled. However, data since 2006 showed that there has been a change in the five most abundant species in the Pascagoula only (*Hybognathes nuchalis* (68.5%), *Cyprinella venusta* (8.5%), *Anchoa mitchilli* (5.9%), *Notropis atherinoides* (5.2%), and *Notropis texanus* (4.4%)), which comprise of about 89% of individuals sampled. These five species in each of the two time periods (historic and contemporary) make up about 90% of total sampled fish in all efforts historically and present. Assemblages within the Leaf and Chickasawhay did not experience a change in fish assemblages. In the Leaf River, both *C. venusta* and *N. longirostris* made up the two most abundant species in both time periods and *P. vigilax*, *Lepomis sp.*, and *A. beani* made up the top five most abundant species in both historic and current samples.

The Chickasawhay River also had *C. venusta* and *N. longirostris* as the most abundant species in historic and contemporary samples (*C. venusta* – 42.0% and 40.9%, *N. longirostris* – 9.5% and 11.8%). Other top five species in historic and contemporary

samples for the Chickasawhay River include *A. beani* and *P. vigilax*. The descriptive statistics calculated include relative and rank abundances of species (Table 2) for each river during historic and contemporary years. This shows that there are common trends in fish assemblages historically compared to what was sampled in current sampling efforts.

In NMDS plots for the Chickasawhay (Figure 3) and Leaf rivers (Figure 4), the fish assemblages are overlapping, indicating similarity between historic and contemporary assemblages. However, in the Pascagoula River (Figure 5), there is less overlap, indicating there is dissimilarity between historic and contemporary assemblages. The Chickasawhay River had a mean Bray-Curtis dissimilarity value of 0.654 within periods, 0.674 between periods, and an overall value of 0.664. The Leaf River had a mean Bray-Curtis dissimilarity value of 0.704 within periods, 0.692 between periods, and an overall value of 0.699. The Pascagoula River had a mean Bray-Curtis dissimilarity value of 0.695 within periods, 0.775 between periods, and an overall value of 0.735.

The results of the PERMANOVA show that there are statistically significant differences in assemblages among periods within each of the three rivers, with the most prominent being in the Pascagoula compared to the Leaf and Chickasawhay rivers (Table 4). Specifically, differences among periods explained 2.5% of the variation in fish assemblages in the Leaf River, 4.5% for the Chickasawhay River, and 41.9% for the Pascagoula River. Thus, variability in assemblage structure between the two periods in the Pascagoula were about ten-times the magnitude in the other two rivers.

1.3.3 Modeling

All three rivers contained contemporary assemblages that changed more than expected due to random baseline variation; however, the proportion of samples varied

among rivers. Only 1 (6.5%) and 5 (14.5%) of samples in the Leaf and Chickasawhay rivers showed significant change from the historical to contemporary samples. In contrast, 17 sites (24.8%) of samples in the Pascagoula River exhibited greater change than expected due to natural background variability. I also calculated the mean contemporary assemblage Euclidean distance from historic assemblage distance and found that the Leaf was $0.08(\pm 0.017)$, the Chickasawhay was $0.10(\pm 0.31)$, and the Pascagoula was $0.39(\pm 0.18)$. Overall, the percentage of significantly different assemblages were almost twice as high in the Pascagoula compared to the Chickasawhay and almost four times as greater than in the Leaf River.

1.4 Discussion

The objectives of this study were to analyze the community composition of the Pascagoula, Leaf, and Chickasawhay rivers to compare historical and contemporary community. I observed change in the top five most abundant taxa only within the Pascagoula River, while the Chickasawhay and Leaf rivers did not experience such dramatic changes in community. I used two long-term datasets to address whether there has been a change in fish community in the Pascagoula River. First, I used the Ratio dataset to calculate the ratio of *H. nuchalis* and *C. venusta* to show that these community patterns were consistent before and after the community shift around 2005. Based on these data, I hypothesize that the shift in assemblages likely happened between 2005 and 2006. Schaefer et al., (2006) studied the Pascagoula River and neighboring tributaries to assess the effects of Hurricane Katrina. The study found that in the years directly after Hurricane Katrina (2005), *H. nuchalis* increased in abundance, contrasting before the Hurricane when their abundances were drastically lower. There are only two samples from 2007-2010 (both are

in 2008). Those samples did have a higher number of *C. venusta* than *H. nuchalis*, therefore Figure 2 indicates that there was a higher proportion of sampled sites that have a greater ratio of *C. venusta* than *H. nuchalis*. But because of the lack of proper sampling, it cannot be concluded that there were more *C. venusta* than *H. nuchalis* within that year and I can infer that during 2007-2010 there were similar trends in community structure to what is observed today. In 2011 when robust sampling continued, the same trends in community samples are observed that are similar to what Schaefer et al. (2006) found with *H. nuchalis* increasing in abundance post Hurricane Katrina. In all sampled years following 2006, *H. nuchalis* is the most dominate species, and that pattern continues into the most current sampling years. Finally, I used the Assemblage dataset to address whether there was temporal variation in assemblages over time within each of the three rivers that could yield changes in composition through normal stochastic processes. Each sample in the Assemblage dataset used a standardized gear, sampling effort, and the same site locales were sampled in each river in each sampled year. If I had a more complete long-term dataset, I would be able to most obviously pinpoint the exact time frame of the assemblage shift; however, I think that by analyzing the two datasets, I can accurately assess the fish assemblages within the Pascagoula River.

I believe that these two datasets were able to support that the Pascagoula River may be in an alternative stable state. If that is the case, the Pascagoula River has two (or more) stable states. The first stable state is defined by the historical assemblages where *C. venusta* (61.1%) is the most abundant species followed by the Southern Hogchoker, *Trinectes maculatus* (5.6%), and two other cyprinids, *Notropis longirostris* (4.6%) and *Pimephales vigilax* (4.0%). The second stable state is defined in the contemporary assemblages where

H. nuchalis (68.5%) is the most abundant species, followed by *Anchoa mitchilli* (5.9%), and a new set of Cyprinids, *Notropis atherinoides* (5.2%), *Notropis texanus* (4.4%), and *Macrhybopsis storeriana* (1.8%). In contemporary samples, *C. venusta* was the second most dominant species, but now only comprising of 8.5% of all individuals instead of 61.1% of total individuals as what had been recorded in historic assemblages. The two states appear stable because each state has persisted for multiple generations and do not seem to be changing from one state to another. PERMANOVA results show that variation between historic and contemporary assemblages is significant, with the two time period explaining almost ten times as much variance in assemblages within the Pascagoula than the other two rivers. I believe that the Pascagoula River is in an alternative stable state rather than other hypotheses describing state shifts. Matthews and Marsh-Matthews' "loose" equilibrium concept (LEC) shows there should be no directional trajectory and no community changes to push into an alternate state (Matthews and Marsh-Matthews, 2016). Matthews and Marsh Matthews (2016) saw that the dynamics of stream fish assemblages of Brier Creek, Oklahoma were consistent with the LEC (Matthews et al. 2013) and repeatedly returned toward average positions in ordination space, or about an equilibrium point. If the Pascagoula were in LEC, there would be community trends that return to an average community composition, i.e. back to the *C. venusta* dominated state. Instead, I see two distinct assemblages that have each persisted over multiple fish generations, with no return to an average equilibrium point. NMDS results show that there was distinct separation in the historic assemblages from the contemporary assemblages, with contemporary assemblages consistent since at least 2011.

With my null model I asked the question of whether the change in fish assemblages within the Pascagoula River could have happened through stochastic processes alone. I found that in the Pascagoula River, contemporary assemblages were more different than historic assemblages based on what would be expected just through natural variation within the system. The null model built in expected natural stochasticity based on the mean abundance and coefficient of variation in abundance over time in historic assemblages. Because of this, I can conclude that there may be some event that caused the historic assemblages within the Pascagoula River to be different than what is expected. The null model and PERMANOVA results indicated that there were differences in assemblages within the Leaf and Chickasawhay rivers; however, these variances are most likely due to natural fluctuations within assemblages rather than driven by an assemblage change and state shift like what I am proposing in the Pascagoula River. The PERMANOVA further supported the hypothesis that there are statistically significant differences between contemporary and historical assemblages within the Pascagoula River.

There are ecological differences in the biology of the fishes that are most abundant in historic assemblages versus contemporary assemblages. A shift in environmental conditions as the result of a disturbance could have allowed some species to be most successful after the state was pushed into its alternative state. *Hybognathus nuchalis* and *C. venusta* saw the most drastic changes in relative abundances during the two time periods and that may be the result of their ecologies. Ross (2001) noted in his field observations that *C. venusta* may be less tolerant of hypoxic conditions, while *H. nuchalis* can occupy low dissolved oxygen (DO) conditions. Those observations were supported by experimental data demonstrating higher DO tolerance of *H. nuchalis* than *C. venusta*

(Barrett, 2019). There is a lack of knowledge on the DO tolerance of the other species that are most abundant in our contemporary assemblages, but I can infer from their habitats that those species may also have a wide DO tolerance range. *Notropis texanus* can be found natural oxbows where oxygen may be lower (S. T. Ross and Baker, 1983) and primarily feed on detritus like *H. nuchalis* (J. A. Baker and Ross, 1981). While *N. texanus* increased in abundance after Hurricane Katrina, Van Vracken and O'Connell (2010) found that *N. texanus* was absent from sites in Bayou Lacombe, LA after the hurricane. Van Vracken and O'Connell (2010) hypothesized that it was the lack of ability to tolerate changing DO conditions that lead to the absence of this species from the area. Further experimental designs could be performed to test this DO tolerance hypothesis of *N. texanus*, as well as exploration of other ecological factors for the increased abundance in my study compared to the absence in the Bayou Lacombe study. Other cyprinids that increased in contemporary assemblages in the Pascagoula may also be hypothesized to be adaptive of their changing environment. *N. atherinoides* will shift diets depending on seasonality and may feed on detrital matter in fall months (Whitaker, 1977). With the increase of fishes that feed on detritus, there could have been a shift in food base for cyprinids with a higher amount of detritus in the environment.

DO tolerance is of interest because Hurricane Katrina could have been a driving factor pushing the community from one stable state to another. Hurricane Katrina was a category five storm that impacted Southeastern United States in August 2005. As the result of large storm events, storm surges bring an influx of saltwater, debris, stirring of anoxic sediments, and fish kills resulting in reduced DO levels in effected areas (Mallin et al. 1999; Buck 2005; Schaefer et al., 2006; Van Vrancken 2010; Dolloff et al., 1994). Multiple

studies reported the effects of Hurricane Katrina and specifically found that in Bayou Lacombe, LA, there were changes to freshwater fish assemblages upstream and downstream of the Bayou (Van Vrancken and O'Connell, 2010). Similarly, Schaefer et al. (2006) documented changes in fish assemblages in 2006 in portions of southeastern Mississippi, including the Pascagoula River, that were impacted by the storm. *Hybognathus nuchalis* increased in abundance after Hurricane Katrina, when before the hurricane when their abundances were minimal. Possible impacts to fish assemblages could have been from destruction to habitats and inability to access refuge habitat. After Hurricane Katrina, the Pascagoula River was pushed into an alternative stable state configuration while the Bayou Lacombe eventually returned to community compositions similar to before the storm. While it is not clear why the Pascagoula River was more impacted compared to the Bayou Lacombe, one hypothesis might be that there may not be an alternative stable state assemblage in Bayou Lacombe.

Anthropogenic and natural disturbances can cause community shifts in freshwater fish assemblages. Aside from hurricanes, droughts are also natural disturbances that can cause changes to fish assemblages. In 2006-2008, the Pascagoula River experienced drought years with Palmer Drought Severity Index of -2.29, -2.75 and -1.17 respectively (NOAA, 2018). With lowered dissolved oxygen levels immediately after the hurricane, as well as three consecutive drought years, conditions could have been more favorable for species like *H. nuchalis* and *N. texanus* than *C. venusta* and *N. longirostris*. The ability to tolerate lower dissolved oxygen concentrations was not only advantageous for overall survival, but post-Katrina species might have been able to seek refuge or avoid predation in deeper pools where dissolved oxygen concentrations were likely lower. Similarly,

Matthews and Marsh-Matthews (2007) reported the rapid decline of a cyprinid species, *Cyprinella lutrensis*, in the Oklahoma-Texas region, where there was no obvious habitat change. They hypothesized that it was continuous droughts that had occurred within the region, coupled with the inability to recolonize because of a local reservoir, that led to the drastic decline *C. lutrensis*. During drought events, the minnow had to seek refuge in deep pools that were home to predators such as sunfish and bass (Matthews and Marsh-Matthews, 2007). The system saw a steep decline (and almost extirpation) of *C. lutrensis*, similar to what is being recorded in the Pascagoula River. Hurricane Katrina could have been the initial disturbance that disrupted the Pascagoula River and coupled with consecutive years of drought could have switched the system from one stable state into an alternative. However, Matthew and Marsh-Matthews (2007) did not observe a whole assemblage change, but rather just a decline in *C. lutrensis*.

Understanding community dynamics and the mechanisms behind how and why assemblages change has been a topic of interest for fish ecologists for decades. The conditions of the Pascagoula River make the system very pristine with little anthropogenic impacts to the natural flow of water to the system. Therefore, by recognizing that there is a change in the fish assemblages within the river, other anthropogenic assessments such as land use, effluents discharge, and effects of urbanization need to be further studied to determine if the ecological resilience (and resistance to disturbances) of the Pascagoula River has worsened. Similarly, further monitoring projects should continue to study the fish assemblages within the river to see if trends in community composition continues or if there is a return back to the previous stable state. There are multiple fish species of interest within the Pascagoula River, such as the endangered Pearl Darter and Gulf

Sturgeon. Therefore, further assessment of the changing assemblages within the Pascagoula River could have implications to these already threatened species. It is with long-term monitoring and data collection that large-scale community changes can be observed, such as the one seen in the Pascagoula River, MS.

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1.6 Tables

Table 1.1

Number of sites sampled per river in historic and contemporary time periods used in both Ratio and Assemblage datasets

Period	Historic (1976-2004)	Contemporary (2010-2019)
Chickasawhay	94	117
Leaf	176	99
Pascagoula	67	120

Table 1.2

Proportion and ranks for the 15 most numerically dominant species in historic versus contemporary assemblages in the Leaf, Chickasawhay, and Pascagoula Rivers

	<u>Leaf</u>		<u>Chickasawhay</u>		<u>Pascagoula</u>	
	<u>Historic</u>	<u>Contemporary</u>	<u>Historic</u>	<u>Contemporary</u>	<u>Historic</u>	<u>Contemporary</u>
<i>Cyprinella venusta</i>	60.7 (1)	60.6 (1)	42.0 (1)	40.9 (1)	61.1 (1)	8.5 (2)
<i>Trinectes maculatus</i>	1.2 (10)	0.8 (14)	2.6 (7)	3.4 (7)	5.6 (2)	1.0 (7)
<i>Notropis longirostris</i>	13.5 (2)	17.2 (2)	9.5 (2)	11.8 (2)	4.6 (3)	0.8 (8)
<i>Pimephales vigilax</i>	3.3 (3)	2.8 (4)	7.4 (4)	4.8 (5)	4.0 (4)	0.5 (11)
<i>Anchoa mitchilli</i>	-	-	-	-	3.0 (5)	5.9 (3)
<i>Carpionodes velifer</i>	-	1.2 (8)	-	-	2.4 (6)	-
<i>Hybognathes nuchalis</i>	-	1.1 (12)	-	8.8 (3)	2.4 (7)	68.5 (1)
<i>Lepomis sp.</i>	3.1 (4)	2.7 (5)	4.7 (6)	4.6 (6)	2.4 (8)	0.2 (15)
<i>Ammocrypta beani</i>	2.6 (5)	3.4 (3)	-	8.5 (4)	2.0 (9)	0.8 (9)
<i>Macrhybopsis storeriana</i>	2.2 (7)	0.9 (13)	6.9 (5)	2.8 (9)	1.9 (10)	1.8 (6)
<i>Notropis vollucellus</i>	2.6 (6)	1.6 (7)	-	2.6 (10)	1.7 (11)	-
<i>Gambusia affinis</i>	0.5 (15)	-	-	-	1.5 (12)	-
<i>Notropis atherinoides</i>	0.9 (11)	1.1 (9)	0.9 (15)	3.2 (8)	1.1 (13)	5.2 (4)
<i>Notropis texanus</i>	-	1.1 (10)	1.6 (10)	1.0 (13)	1.1 (14)	4.4 (5)
<i>Eucinostomus harengulus</i>	-	-	-	-	0.7 (15)	-
<i>Micropterus punctulatus</i>	0.7 (12)	1.1 (11)	1.4 (11)	1.5 (12)	-	0.5 (10)
<i>Lepomis megalotis</i>	-	-	-	-	-	0.3 (12)
<i>Labidesthes sicculus</i>	-	-	-	-	-	0.2 (13)

Table 1.2 continued

<i>Hybopsis winchelli</i>	1.3 (9)	1.9 (6)	1.4 (12)	2.1 (11)	-	0.2 (14)
<i>Hypentelium nigricans</i>	1.7 (8)	-	2.2 (8)	-	-	-
<i>Gambusia holbrooki</i>	-	-	1.6 (9)	-	-	-
<i>Percina aurora</i>	-	-	1.1 (13)	0.9 (14)	-	-
<i>Notropis amplamala</i>	-	-	0.9 (14)	-	-	-
<i>Percina suttkusi</i>	-	-	-	0.5 (15)	-	-
<i>Percina sciera</i>	0.6 (13)	-	-	-	-	-
<i>Lepisosteus oculatus</i>	0.5 (14)	-	-	-	-	-
<i>Lythurus roseipinnis</i>	-	0.7 (15)	-	-	-	-

Table 1.3

ANOVA table with river and period as factors, as well as the interaction of the two, on the relative proportion of H. nuchalis and C. venusta

Source of Variation	Df	Sum of Squares	Mean Squares	F ratio	p-value
River	2	33.38	16.69	305.6	<0.0001
Period	1	10.81	10.809	197.9	<0.0001
River * Period	2	15.87	7.935	145.3	<0.0001
Residuals	741	40.46	0.06		

Table 1.4

PERMANOVA results indicating how much variation is explained by historic versus contemporary assemblages in each river

Pascagoula

	Df	Sum of Squares	Mean Squares	F. Model	R ²	Pr(>F)
Period	1	3.41	3.41	20.35	0.42	0.000999*
Residuals	150	25.15	0.17		0.58	
Total	151	28.56			1.00	

Leaf

	Df	Sum of Squares	Mean Squares	F. Model	R ²	Pr(>F)
Period	1	1.22	1.22	6.68	0.02	0.000999*
Residuals	266	48.65	0.18		0.98	
Total	267	49.87			1.00	

Chickasawhay

	Df	Sum of Squares	Mean Squares	F. Model	R ²	Pr(>F)
Period	1	1.52	1.52	10.09	0.05	0.000999*
Residuals	213	32.06	0.15		0.95	
Total	214	33.57			1.00	

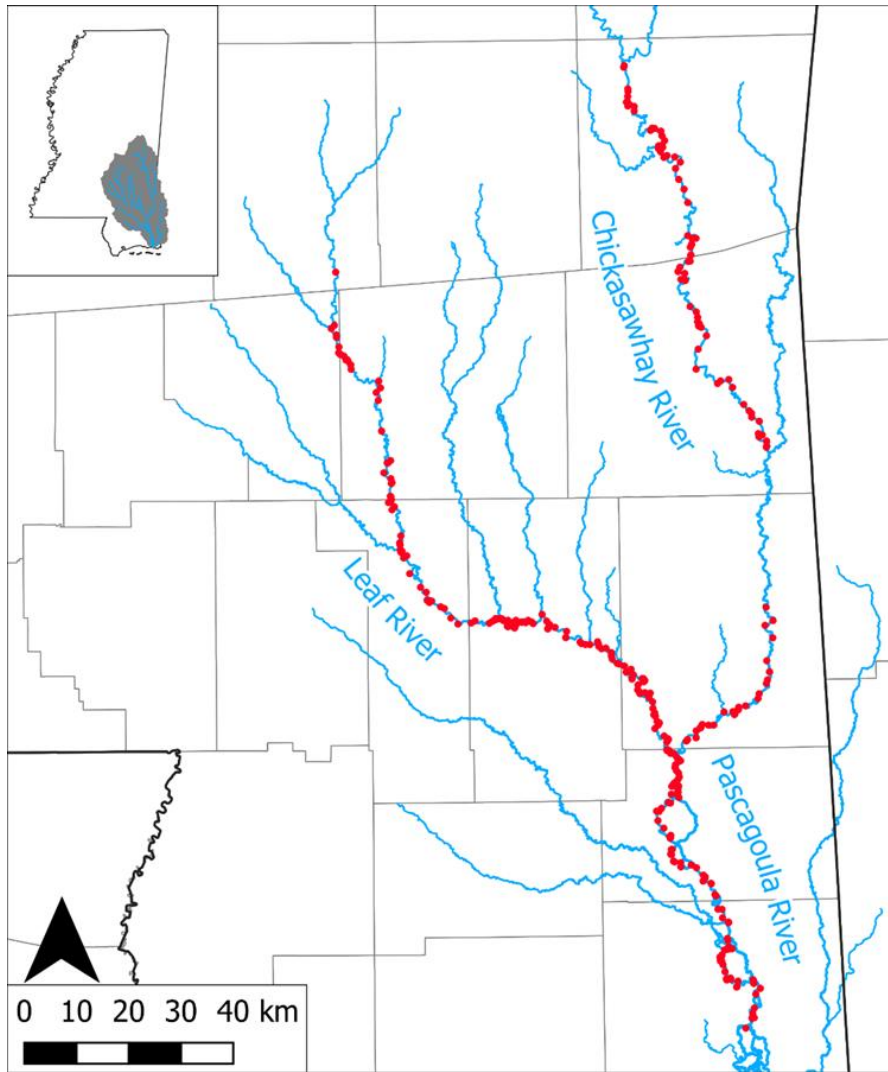


Figure 1.1 *Map of the sample sites*

Note: N = 747 within the Chickasawhay, Leaf, and Pascagoula Rivers throughout the study period (1976-2019)

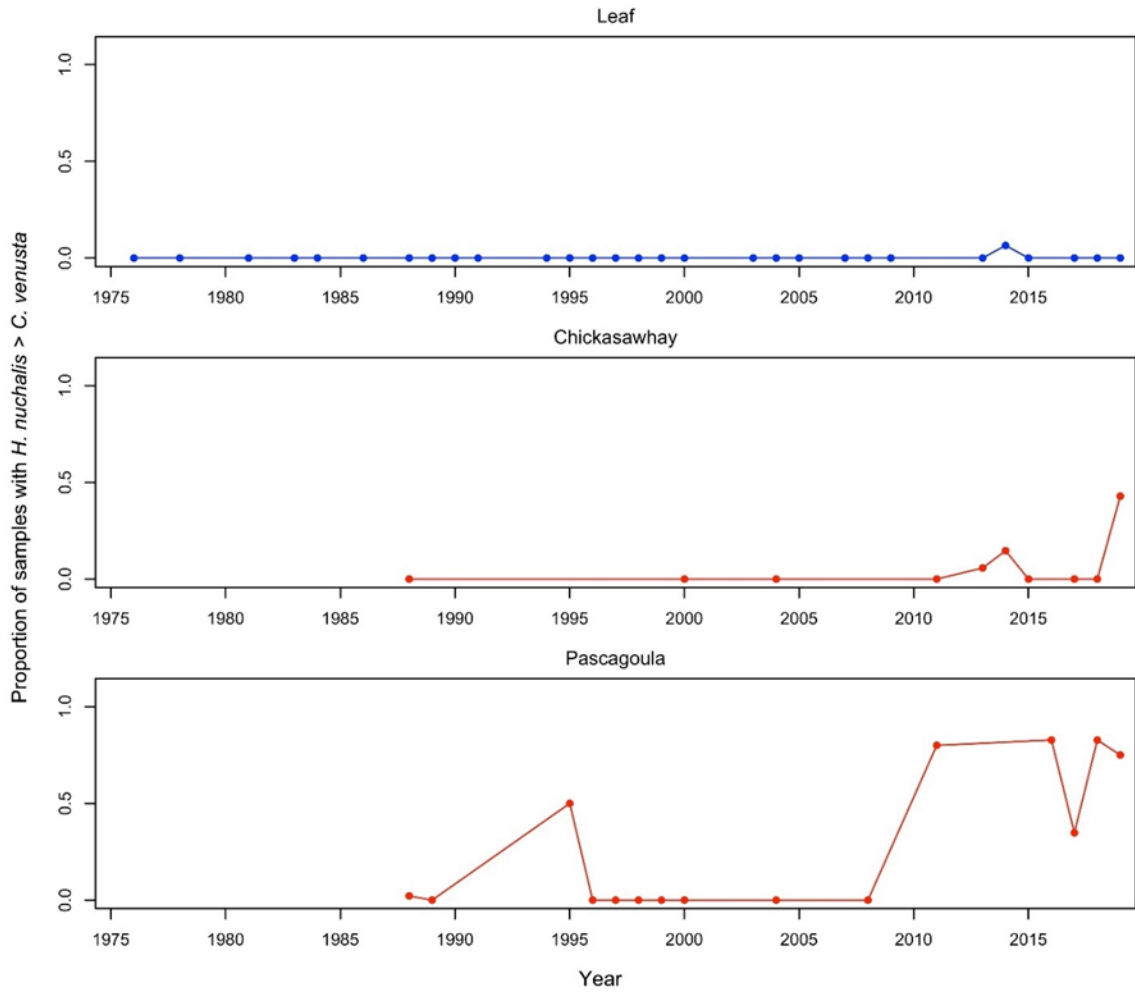


Figure 1.2 *Proportion of samples where there was a higher relative abundance of *H. nuchalis* than *C. venusta* in the Leaf, Chickasawhay, and Pascagoula Rivers*

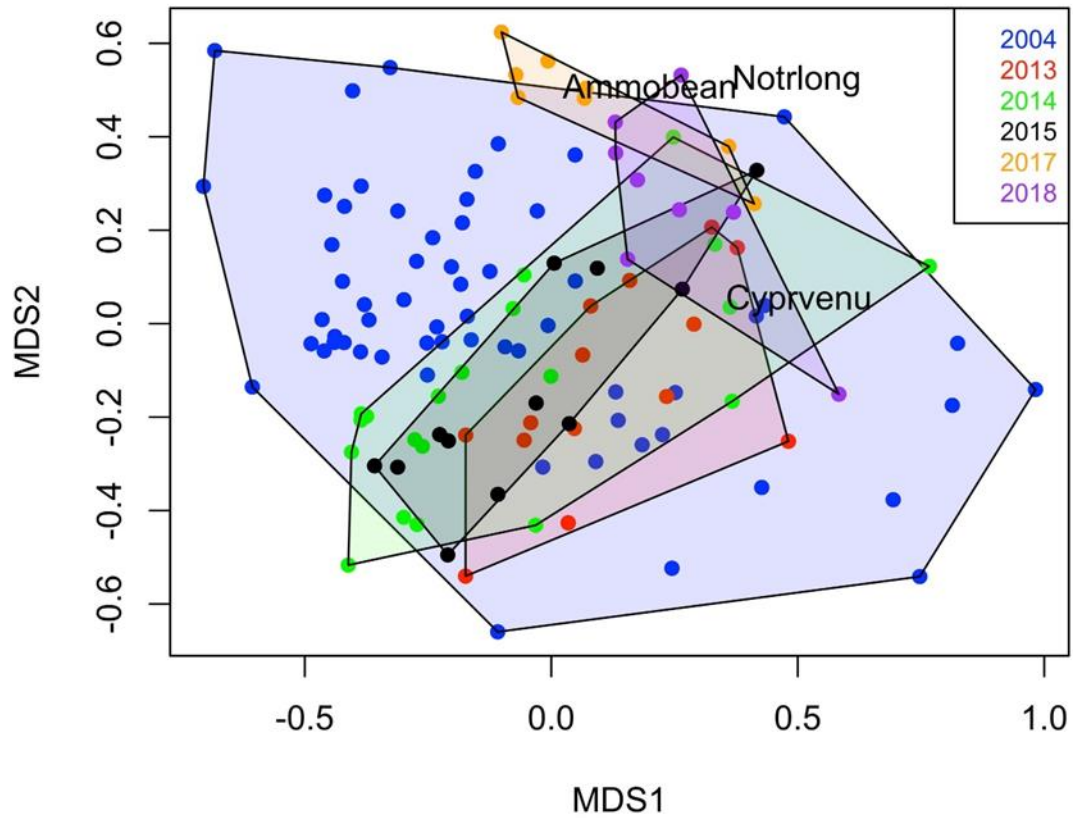


Figure 1.3 Non-metric multidimensional scaling (NMDS) determining assemblage composition for the Leaf River

Note: Different colored polygons represent different sampled years. Species weighted average scores are indicated within the plot (Cyprvenu – *Cyprinella venusta*, Ammobean – *Ammocrypta beani*, Notrlong – *Notropis longirostris*). The NMDS shows that assemblages throughout years are similar due to overlap.

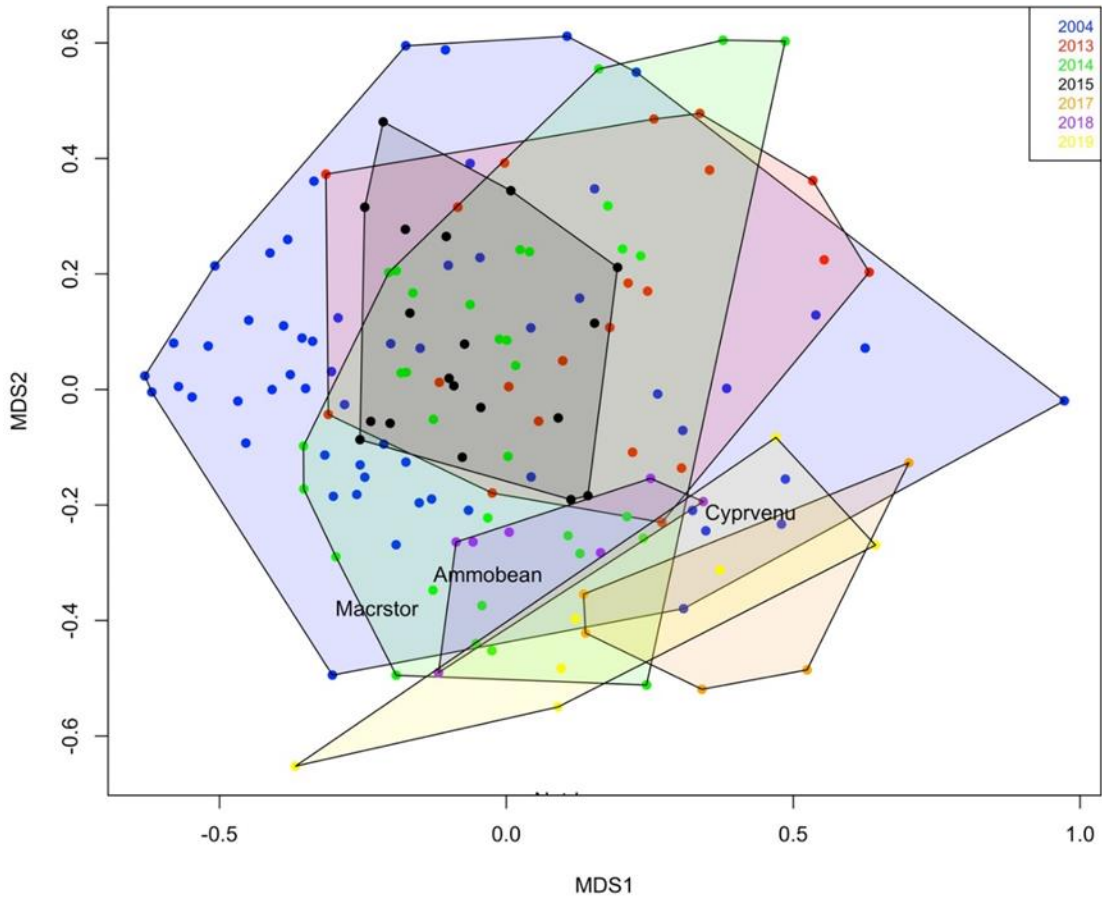


Figure 1.4 *Non-metric multidimensional scaling (NMDS) determining assemblage composition for the Chickasawhay River*

Note: Different colored polygons represent different sampled years. Species weighted average scores are indicated within the plot (Cyprvenu – *Cyprinella venusta*, Ammobeane – *Ammocrypta beani*, Macrstor – *Macrhybopsis storeriana*). The NMDS shows that assemblages throughout years are similar due to overlap.

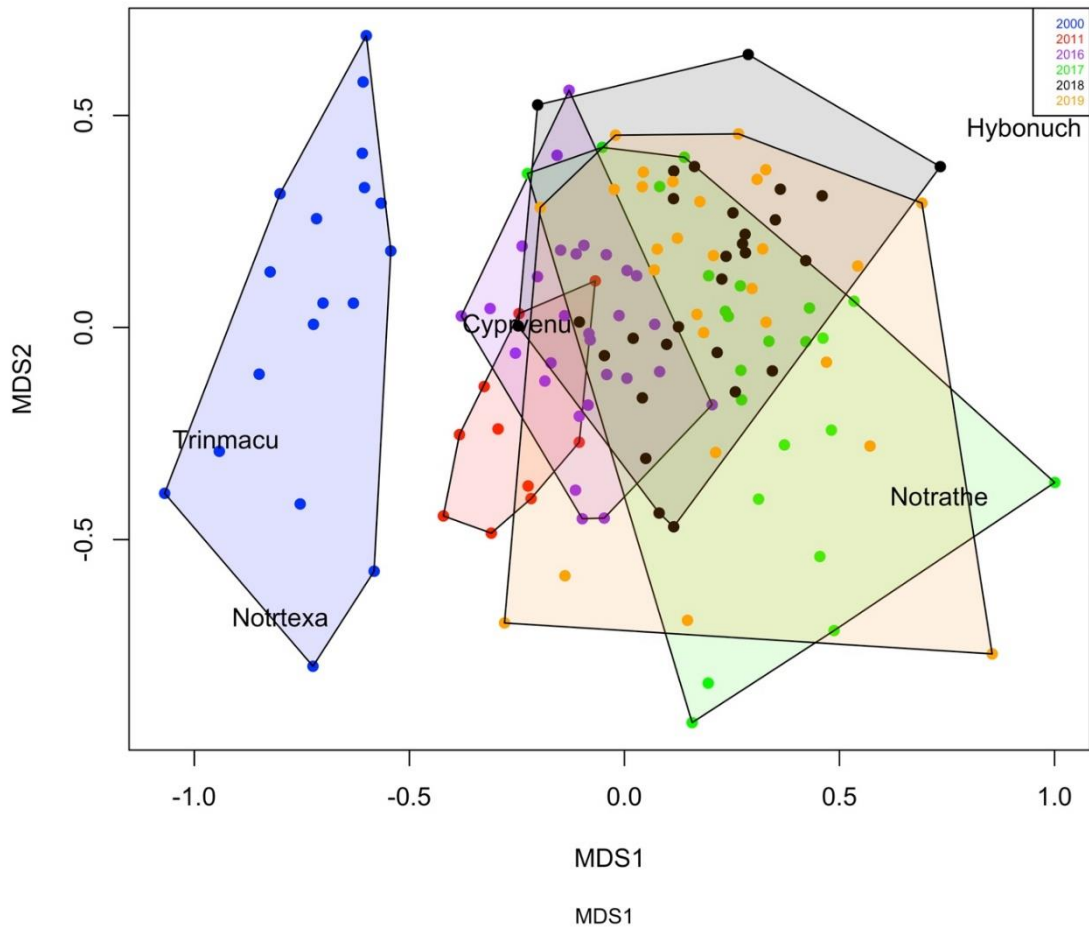


Figure 1.5 *Non-metric multidimensional scaling (NMDS) determining assemblage composition for the Pascagoula River*

Note: Different colored polygons represent different sampled years. Species weighted average scores are indicated within the plot (Cyprvenu – *Cyprinella venusta*, Notttexas – *Notropis texanus*, Notrathe – *Notropis atherinoides*, Hybonuch – *Hybognathus nuchalis*, Trinmacu – *Trinectes maculatus*). The NMDS shows that assemblages throughout years are not similar due to little overlap.

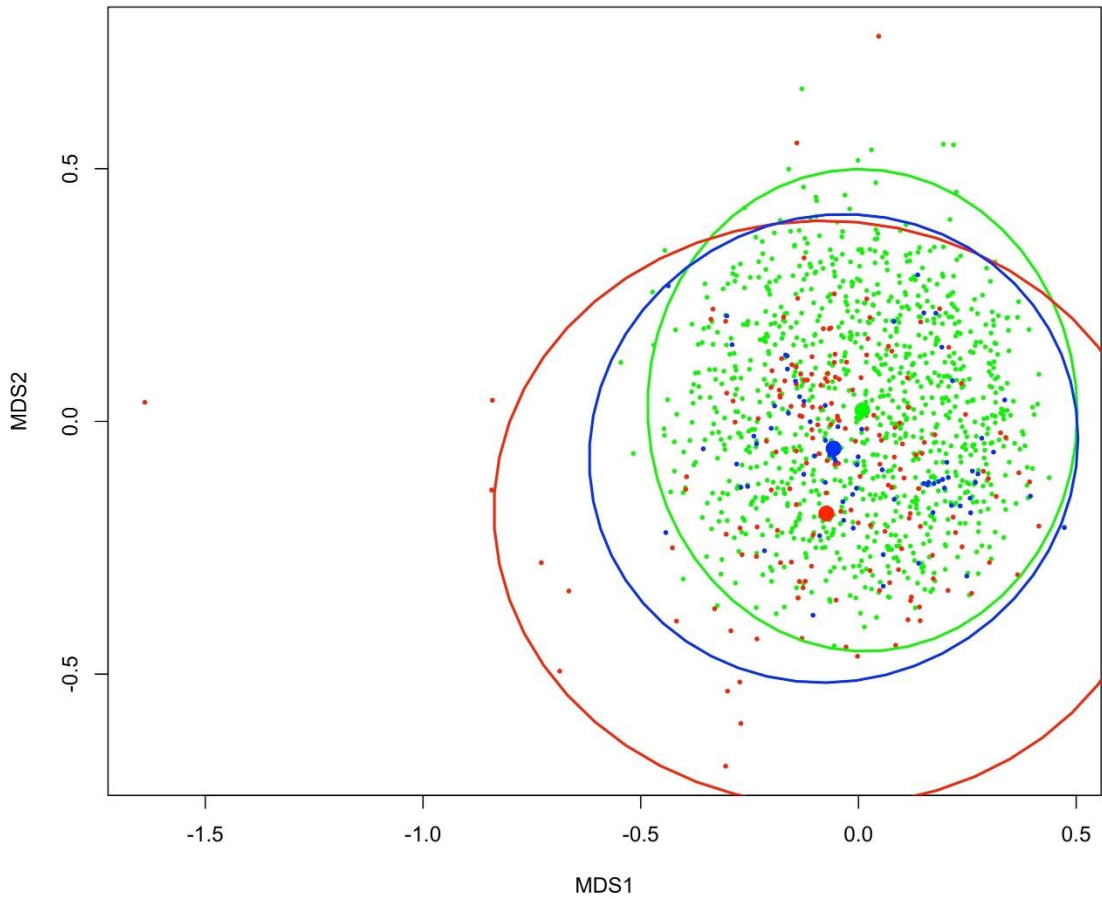


Figure 1.6 *Non-metric multidimensional scaling (NMDS) for the null model of the Leaf River*

Note: Green points represent the randomly generated assemblages, red points represent historic samples, and blue points indicate contemporary samples. Large dot represents the centroid for each of the NMDS group of points. Circles represent 95% confidence intervals.

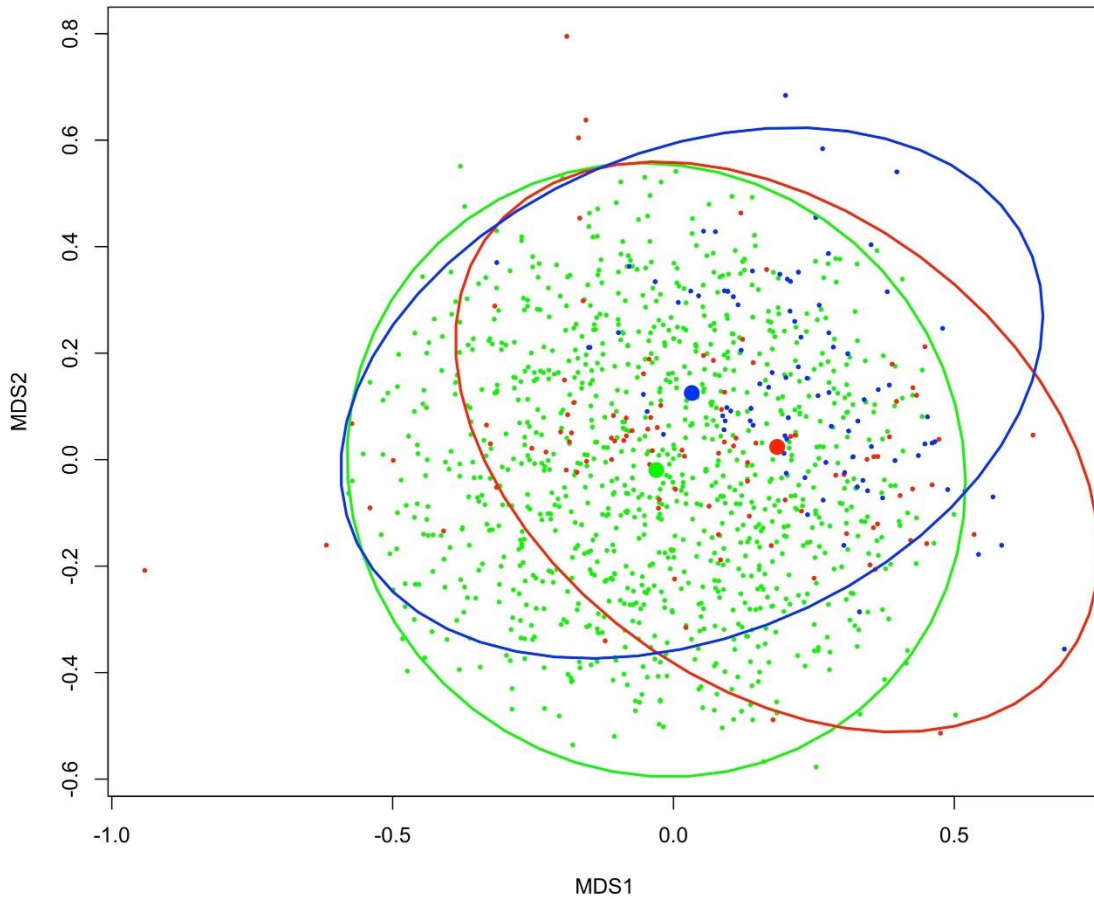


Figure 1.7 *Non-metric multidimensional scaling (NMDS) for the null model of the Chickasawhay River*

Note: Green points represent the randomly generated assemblages, red points represent historic samples, and blue points indicate contemporary samples. Large dot represents the centroid for each of the NMDS group of points. Circles represent 95% confidence intervals.

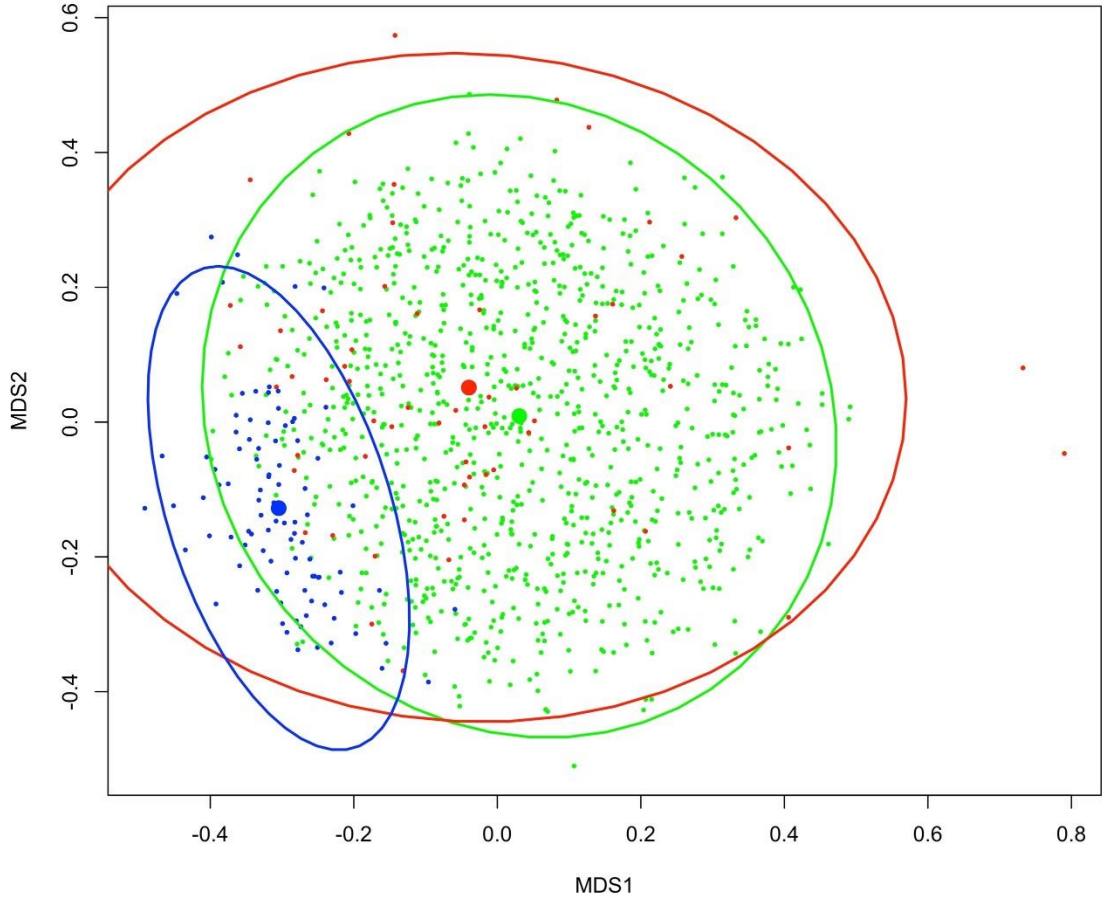


Figure 1.8 *Non-metric multidimensional scaling (NMDS) for the null model of the Pascagoula River*

Note: Green points represent the randomly generated assemblages, red points represent historic samples, and blue points indicate contemporary samples. Large dot represents the centroid for each of the NMDS group of points. Circles represent 95% confidence intervals.

CHAPTER II – DISSOLVED OXYGEN TOLERANCE OF TWO CYPRINID SPECIES

2.1 Introduction

Physiochemical properties are important drivers of freshwater fish assemblages. Temperature, pH, and dissolved oxygen (DO) have all been linked to population abundance or assemblage composition of fishes and have been studied since the early 20th century (Grinnell, 1917; Andrewartha and Birth, 1954). Anthropogenic acidification of streams and lakes have proven to be detrimental for fish populations, as fish are poor regulators of internal pH and can become stressed in unfavorable conditions (George et al., 2019). Variation among assemblages in freshwater systems can be related to species tolerances or physiochemical optima along DO, temperature, or other gradients which fluctuate seasonally and geographically (Gelwick et al., 2001; Matthews and Marsh-Matthews, 2017). Drastic changes in DO concentrations have been documented to cause changes in distributions or abundances of aquatic populations (Fry 1971; Summers et al., 1997; Diaz and Rosenberg, 1995; Lerberg et al, 2000; Wenner et. al., 2004). There are notable impacts of lower DO to changes in fish communities, but these are considered short-term as the community eventually shifts back to pre-disturbance conditions (Dolloff et al., 1994 and Stevens et al., 2006). Thus, understanding DO fluctuations are an important part of water-quality assessments and monitoring because oxygen tolerances can drive changes to fish distributions and abundances.

Dissolved oxygen concentrations can be quite dynamic in rivers and streams. DO concentration in the water is a function of primary production, exchange with atmospheric air, and respiration or biological oxygen demand (BOD). Rates of productivity and respiration are driven by nutrient availability, temperature, light (and its transmission

through water), and other environmental conditions (Odum 1956; Stumm and Morgan 1996; Venkiteswaran et al., 2007). Biological oxygen demand (BOD) is the amount of dissolved oxygen needed for metabolic activity of aerobic organisms (Udeigwe and Wang, 2010; Preininger et al., 1994). DO is consumed by bacteria when large amounts of organic materials are in the water in addition to algae respiration and breaking down photosynthetic products. Oxygen concentrations change along the river continuum as organic input is sourced from coarse particulate organic matter, such as fallen leaves, in the smaller headwater streams to finer particulate organic matter in larger, downstream rivers (Vannote et al., 1980). Overall, oxygen in rivers and streams is produced by photosynthesizing algae, while it is consumed by respiration, BOD processes, and sediment oxygen demand and oxidation. Simultaneously, the aquatic systems can be re-aerated through the exchange with atmospheric air (facilitated by higher turbulent flows). When DO consumption exceeds DO supply from photosynthesizing algae and exchange with the atmosphere, DO levels drop.

As a result of changes to the abiotic environment, fish have developed physiological and morphological adaptations to dealing with harsh DO conditions. Most fish avoid hypoxic waters, but many species can tolerate short periods of detrimentally low DO conditions (Rahel and Nutzman, 1994). Such adaptations include large gill surface area, low metabolic rate, and morphological specializations for utilization of oxygen-rich surface layer (Galis and Barel, 1980; Kramer, 1983; Perry and McDonald, 1993). Another adaptation is the use of aerial surface respiration (ASR) that is characterized by when an individual uses the air-surface interface in water with low oxygen concentrations (Kramer, 1983a). Ultimately, mortality may result from hypoxia events, depending on how low the

concentrations are and exposure time to hypoxic waters. Tolerances can differ among species, life stages, and seasonally (Sylvester et al., 1975; Burton et al., 1980; Coutant, 1985; Love and Rees, 2002; Wannamaker and Rice, 2003). Responses to DO are shaping the evolution of fish species, as populations and species are adapting different responses to low DO conditions. Because of this, the effects of abiotic disturbances, specifically fluctuations in DO, and how it alters freshwater fish communities and species abundances is still an important, underlying question for aquatic ecologists (Matthews 1998, Pollock et al., 2007).

Both anthropogenic and natural disturbances can cause DO levels to change in aquatic systems. Storms and hurricanes are natural disturbances that can increase the disruption of anoxic sediments and can create short-term anoxic or hypoxic conditions in estuaries and intertidal zones, with the potential to have effects in nearby rivers (Mallin et al., 1999; Buck, 2005). The effects of storms and hurricanes on DO levels have been studied since the 1960s (Tabb and Jones 1962). In rivers and streams, storms can increase input of allochthonous materials as leaf litter and debris falls into the streams. This can result in increased BOD as the organic material decomposes, causing overall poorer water quality for stream fishes (Knott and Martore, 1991; Paerl et al., 2001; Burkholder et al., 2004). Anthropogenic disturbances can cause low DO concentrations in freshwater systems through nonpoint and point pollutions and is considered one of the most disruptive and dangerous water quality problems (Goldberg, 1995). Nutrient overloading causes eutrophication and BOD consumes available oxygen from the system. Water column stratification inhibits deep water mixing with water from the surface, resulting in an anoxic condition that can be harmful to aquatic organisms. While the mechanisms on how

dissolved oxygen fluctuates in aquatic systems is well understood, there is still more to be understood about how DO tolerances can affect stream fishes and assemblage dynamics. There is a lack robust measures of DO tolerances for most of the abundant stream fishes which prevents proper understanding of how low DO might impact freshwater assemblages.

I am interested in understanding the DO tolerances of two minnow species (*Cyprinella venusta* and *Hybognathus nuchalis*), because there has been a shift in the relative abundance of these two fishes over time in the Pascagoula River, MS. The Pascagoula River basin contains the Leaf and Chickasawhay rivers, which combine to form the Pascagoula River. The Pascagoula River is the largest unimpounded river remaining in the continental United States (Dynesius and Nilsson 1994). The relatively pristine conditions of the Pascagoula River make it ideal for studying effects of disturbance on fish community dynamics.

Long-term fish assemblage data (1976-2019) from the Pascagoula River Basin indicate *C. venusta* was historically (1976-2004) the most abundant species. However, since 2006, *H. nuchalis* seems to have replaced it as the most abundant species in the Pascagoula River. Prior to 2006, *H. nuchalis* had low overall abundance in the Pascagoula River (2.5% relative abundance) and Schaefer et al. (2006) noted an increase in *H. nuchalis* immediately following Hurricane Katrina in August 2005. This trend appears to have persisted within the Pascagoula River, as the percentage of samples dominated by *H. nuchalis* increased from 2.5% to 64.2% in current samples (about 18 individuals per sample historically to 170-3500 individuals per sample in current samples). Whereas abundances

for *H. nuchalis* in the Leaf and Chickasawhay rivers has consistently remained below 8% throughout all sampled years.

Ecologically, *H. nuchalis* has been thought of as tolerant of low oxygen conditions and is found to inhabit areas where DO may be lower (i.e. oxbows and backwaters), while *C. venusta* is less tolerant of low oxygen conditions (W. J. Matthews 1987; Ross 2001). In an experimental study, Matthews (1987) determined that *C. venusta* had 100% mortality rate after 8 hours when in DO concentrations were between 0.2-0.9 ppm. Whitworth (1961) studied DO tolerances of *H. nuchalis* and found that individuals could survive in DO less than 1.0 ppm and 20% of individuals survived in DO tolerances less than 0.2 ppm.

One common study design to assess DO tolerance uses test chambers, or experimental tanks, described by Hlohowskyj and Wissing (1987) and Gilmore et al. (2017), through which nitrogen gas is bubbled to reduce dissolved oxygen (Richards, 1977; Matthews, 1987; Ostrand and Wilde, 2001). The experimental tanks can either be set to predetermined oxygen concentrations where mortality is assessed after a predetermined time frame (Breitburg, 2002; Dixon et al., 2017) or can drop at a consistent rate (Smith and Able, 2003; Schofield et al., 2007) to determine oxygen tolerance. Studies can either report survivorship at set DO concentrations or can specifically calculate the lethal concentration where 50% mortality is observed (LC50; (Smith and Able, 2003; Boyd, 2005). Because of the difference in methodologies and a lack of tolerance data for most of the common Southeastern US freshwater fishes, results are not directly applicable and comparisons between studies can be complicated. The objective of this study is to determine the DO tolerance of *C. venusta* and *H. nuchalis* using lethal oxygen concentration (LC50) and species first use of ASR.

2.2 Methods

2.2.1 Study Area

Cyprinella venusta and *H. nuchalis* were caught using a double lead line, 2.4 × 9.1 m (3.2 mm mesh) seine in the Pascagoula River near Benndale, MS (site coordinates: 30.801367N, -88.727217W). Fish were transported to the University of Southern Mississippi wet laboratory and housed in 500 L stock tanks (two per species) until the start of the experimental trials. All four stock tanks were kept under the same set-up with a mechanical and biological filtration system using a sump tank. The four stock tanks and six experimental tanks (set-up explained below) had water physicochemical parameters similar to typical conditions in the Pascagoula River during summer months (DO = 8.0 +/- 0.30 mg/L; conductivity = 750-850 µS/cm; 24-25°C; total dissolved solids = 68-70 mg/L; pH = 6.15 +/- 0.02). Total dissolved solids (TDS) and pH were measured once in the stock tanks and were similar to the measurements in the experimental tanks, so I inferred that pH and TDS conditions were consistent throughout. All tanks were subjected to a 14-hour light:10-hour dark daily photoperiod. Fish were fed freeze-dried bloodworms and frozen brine shrimp daily. All procedures were performed under IACUC approval (IACUC approval number 18121303).

2.2.2 Experimental Design

For each trial, six 37.5 L experimental tanks were set up following a similar design of Gilmore et al. (2017) (Scheme 1). Tanks were set up on a continuous flow-through filtration system with the sump tank and was cycling at all times, except during experimental periods.

To begin each trial, twenty fish were selected randomly from the stock tanks and placed in one of six experimental tanks (three tanks per species; n = 60 individuals for *C. venusta* and 60 individuals for *H. nuchalis*). The fish were acclimated to their experimental tanks for 24 hours, during this time fish were starved. After this period, flow through tanks stopped and trials began. I covered each tank with Saran Wrap® and sealed with Duct Tape® to minimize interaction with atmospheric air. Nitrogen (N₂) gas was bubbled into each of the six experimental tanks at a rate that lowered oxygen concentrations approximately 1.5 mg/L per hour down to 0.20 mg/L after approximately six hours but no more than eight hours (Figure 1). I conducted pilot trials to determine how much N₂ to introduce to the flow through system to ensure that DO declined at that constant rate; those trials were successful and were added to the analyses. I chose 0.2 mg/L as an ending concentration because it was difficult to reach an oxygen concentration lower than this level and it was a sufficient concentration to quantify LC50 (mortality rates were typically high by this point).

During the trials, DO concentrations were recorded hourly (YSI Professional Plus) in each of the six tanks. There was one tank that was randomly selected in each trial where DO was logged every fifteen minutes. Throughout the trials I observed and recorded when an individual first used ASR. I also recorded fish mortalities indicated by loss of righting response and lack of opercular pumping. If mortality was observed, the fish was immediately removed from the experimental tank, measured (standard length in millimeters (mm)), and DO concentration was recorded. The trial was terminated when 100% mortality was reached in the tank or the oxygen concentration was 0.20 mg/L (+/- 0.02 mg/L).

In order to address the overall question of whether there was a difference in dissolved oxygen tolerances between our two species, I first analyzed whether there was an effect of size (mm) on DO tolerance. I ran an analysis of covariance (ANCOVA) with size (mm) as a covariate and the oxygen concentration at mortality as the response variable for each species. For analyses, I log transformed DO concentrations at mortality because DO concentrations did not adhere to a normal distribution. Individuals in this analysis are considered pseudo replicates as I am treating each individual separately to specifically test for the effects of individual size. There was lack of significance of size of individuals by tank, so I proceeded with using tanks within a trial as the unit of replication to test for species differences in DO concentration tolerance ($n = 12$ for *C. venusta* and $n = 10$ for *H. nuchalis*).

For each tank replicate, I fit a model to interpolate the dissolved oxygen levels at 50% mortality (LC50). These LC50 values served as the response variable in an analysis of variance (ANOVA) testing for a difference in the LC50 between the two species. I used the relationship between DO and survivorship and a 2-parameter non-linear least squares regression to fit a curve to interpolate the dissolved oxygen levels at 50% mortality (LC50) for each tank during each trial. To assess the LC50 values, I calculated the DO concentration where 50% survivorship ($y=50$) occurred based on our non-linear regression model (Figure 2). Once LC50 values were determined for each tank-trial combination, I used an ANOVA to test for the difference in LC50 between the two species. I also ran an ANOVA to test whether there is a difference in the DO levels that both species begin ASR among each tank.

2.3 Results

2.3.1 Summary of Experimental Design

For data analysis, there were four trials completed that lasted no more than eight hours. Within those four trials, there were twenty-two tank replicates that each contained twenty fish of one species per tank (*C. venusta* n = 12, *H. nuchalis* n = 10). There were two instances where some individuals did not survive the 24-hour acclimation period and those tanks had nineteen and eighteen individuals respectively. This did not affect the results of the tank within that trial, and those tanks were not removed from analyses. Overall, there were 239 *C. venusta* and 198 *H. nuchalis* individuals tested. Individual fish sizes varied between 33-68mm in total length. Figure 1 shows the average rate of decline in DO per hour among all trials.

2.3.2 Effect of size on DO tolerance

I tested if there was a significant effect of individual body size (mm) on the DO concentration at mortality between *C. venusta* (n = 239) and *H. nuchalis* (n = 198). I first used an ANOVA to test whether individual body size (mm) varied across trials and found no significant effect ($F_{1,3} = 1.187$, $p = 0.314$) indicating body sizes were similar among trials. I then used ANCOVA to test the effects of species and body size (mm) on DO concentration at mortality. The ANCOVA showed that there was no significant effect of body size on the DO concentration at mortality ($F_{1,1} = 0.238$, $p = 0.626$) (Table 1).

2.3.3 LC50

The ANOVA showed that there was a significant difference in LC50 between *H. nuchalis* and *C. venusta* ($F_{1,1} = 26.032$, $p < 0.0001$) (Table 2). The average LC50 for *H. nuchalis* was 0.93 mg/L (+/- 0.062) and 1.21 mg/L (+/- 0.162) for *C. venusta* (Figure 3;

Table 2). Figure 2 shows the fitted model to predict the LC50 for that particular *C. venusta* tank replicate. The green star indicates the predicted LC50 as determined from the model.

2.3.4 ASR

I observed that *C. venusta* started to use ASR at an average DO concentration of 3.03 mg/L (± 0.051 mg/L, range = 3.39 mg/L – 2.91 mg/L). *H. nuchalis* began using ASR between the fourth and fifth hour when DO concentrations were on average 1.64 mg/L (± 0.26 mg/L, range = 2.28 mg/L – 1.06 mg/L). The effect of species on DO concentration when ASR first began was significant ($F_{1,1} = 400.962$, $p < 0.001$) and there was no significant interaction between species and trial (Table 3).

2.4 Discussion

Our study determined there are differences in DO tolerances of two cyprinid species *H. nuchalis* and *C. venusta*. I was interested in the tolerance of these two species because there has been a drastic shift in the relative abundances of both species in the Pascagoula River. Field observations suggest that *C. venusta* is less tolerant of hypoxic conditions, while *H. nuchalis* can occupy low DO environments (Ross, 2001), but no studies have directly quantified these field observations. Similarly, there is a lack of knowledge in the DO tolerances of many of the most abundant southeastern US minnow species. By understanding the DO tolerance of these two species, there is a stronger understanding of whether differences in physiological tolerances might have been a driving factor in the changes of abundances of these two fishes observed in the Pascagoula River.

In other studies of physiological tolerances among life stages of fishes, it has been shown that juveniles are less tolerant to factors such as temperature, DO, and pH; juvenile fishes have higher metabolic (Moss and Scott, 1961) and growth rates in warmer waters

when oxygen concentrations are near saturation levels (Ruelle, 1971; Matthews and Hill, 1979). The results from this study demonstrated that DO was less detrimental to *H. nuchalis* compared to *C. venusta*; however, our findings did not show an effect of size on dissolved oxygen tolerance between our cyprinid species. A probable reason as to why there was no significant effect of size was that the individuals of the experiment ranged from 33-68 mm in standard length. For both of species, the minimum size in the study indicated that all individuals were at least of reproductive maturity (Heins and Dorsett, 1986). Because all of the individuals were sub adults to adults, juveniles were not tested in the study and I was unable to answer whether there were differences in DO tolerances among life stages. A more robust study should be performed to adequately assess that relationship.

Fishes use a variety of adaptive behaviors when exposed to hypoxic events. These behaviors range from altered swimming activity (quick bursts of increase swim speeds or horizontal swimming), gill ventilation, use of the oxygen rich surface layer at the air-water interface (Lewis, 1970) or storing atmospheric air (Kramer, 1983a; Gee and Gee, 1991; Dean and Richardson, 1999; Mandic et al., 2009). ASR is a commonly observed behavioral response of fishes to severe aquatic hypoxia (Gee et al., 1978; Kramer and McClure, 1982; Rosenberger and Chapman, 2000). I found that *C. venusta* started to perform ASR earlier than *H. nuchalis*. *Cyprinella venusta* began to use ASR at DO concentrations of 3.03 mg/L under conditions of relatively mild hypoxia (~2.50 mg/L) and were not able to survive when waters were severely hypoxic (<2.00 mg/L) or anoxic (<0.5 mg/L). *Hybognathus nuchalis* began to use ASR under severe hypoxic conditions, suggesting greater tolerance to severe hypoxia as these individuals were able to withstand a lower oxygen concentration

before needing to respire at the surface. In a study of hypoxia in tropical freshwater fishes, 29 out of 31 species (94%) performed ASR in DO concentrations between 2.11-2.34 mg/L (Kramer and McClure, 1982). The use of ASR would require greater physiological effort to maintain desired homeostasis and could impair other activities such as reproduction, feeding, and predator avoidance. Because *C. venusta* respire at the surface at a higher DO concentration than *H. nuchalis*, it could make this species more vulnerable to predation and limit other functional behaviors that are pertinent for survival. Because *H. nuchalis* does not need to rely on ASR as quickly as *C. venusta* does, it may be less prone to other sources of mortality such as predation.

Studies have examined DO tolerances for other cyprinid species. The experimental results support field observations of the physiochemical ranges used by these two species (Ross, 2001). Whitworth (1961) has the only other direct study of *H. nuchalis* and dissolved oxygen tolerances. Whitworth (1961) results were similar to the results found in this study, indicating that *H. nuchalis* could tolerate dissolved oxygen concentrations less than 1.0 ppm for over 18 hours. Of the 167 individuals in the Whitworth (1961) study, 35 individuals survived to the end of the experiment with a DO concentration of 0.2 ppm. In another study with a closely related species, *H. placitus*, Ostrand and Wilde (2001) found that the LC50 for this species was also comparable at 2.08 mg/L, ± 0.14 mg/L). *H. placitus* was able to withstand DO concentrations less than 1.0 mg/L for 18-26 hours, but only when given access to surface waters to perform ASR. When not given access to the surface (i.e. placed in a test chamber within the experimental tank), survivorship was anecdotally noted to be lower (Doudoroff and Shumway, 1970; Whitworth and Irwin, 1964). I allowed access to the surface in the study; however, the surface air was likely similar in DO concentration

because I placed Saran Wrap® over the tanks. *Hybognathus nuchalis* still attempted to perform ASR and despite being able to perform ASR, eventually died at the end of the trial (approximately six to seven hours). If Saran Wrap® was not placed over the experimental tanks and fish had exposure to atmospheric air, I hypothesize that *H. nuchalis* would also be able to maintain survivorship in DO concentrations less than 1.0 mg/L for a greater period of time (longer than the eight hours in my study) based on the findings of Whitworth (1961) and similarities in DO tolerances as closely related *H. placitus*. As previously mentioned, experimental designs of DO tolerances vary, so it is difficult to directly compare one study to another. A more standardized design would aid in directly comparing DO tolerances between studies.

I am exploring these tolerance differences as a potential mechanism to explain the changes in relative abundance of these two species in the Pascagoula River. One hypothesis on the mechanism behind the switch in abundances is the implications of Hurricane Katrina. Hurricanes have posed dangerous threats to fish assemblages in coastal regions of the Southeastern United States. Saltwater surges from storms produce drastic turbulences that can lead to direct fish mortalities, but also disturb anoxic sediments that can create short-term anoxic or hypoxic conditions (Mallin et al. 1999; Buck 2005). Storms also produce debris dams, composed of uprooted trees and large quantities of riparian defoliation (Dolloff et al., 1994; Schaefer et al., 2006; Van Vrancken and O'Connell, 2010). The debris dams concentrate leaf litter into confined areas and the increase in detritivore activity leads to decreased oxygen concentrations (Hill et al., 1998). Hurricanes can disturb large quantities of coarse particulate organic debris in smaller headwater streams that could make its way into larger rivers downstream as fine particular organic

matter and alter DO concentrations in rivers as suggested by the River Continuum Concept (Vannote et al., 1980). Hurricanes can drop DO levels in affected areas to below 2.0 mg/L (Tomasko et al., 2006, Mallin et al. 2002, Mallin and Corbett, 2006, Van Vrancken and O'Connell, 2010) that can persist for months after the storm (Tomasko et al. 2006). Schaefer et al. (2006) showed changes in fish communities after Hurricane Katrina in the Pascagoula River, but not in the Leaf and Chickasawhay Rivers, nor a neighboring tributary of Black Creek, MS. Specifically Schaefer et al (2006) saw an increase in *H. nuchalis* after Hurricane Katrina. Similarly, Van Vrancken and O'Connell (2010) observed that dissolved oxygen concentrations after Hurricane Katrina led to shifts in freshwater fish assemblages in both up- and downstream reaches of Bayou Lacombe, LA. Species that were most successful immediately post-storm consisted of species that were more tolerant of concentrated organic debris and lower oxygen concentrations, as well as species that were able to seek refuge from downstream storm surges by moving into upstream habitats. Downstream communities were most affected as debris dams and riparian defoliation allowed detritivores to thrive. Schaefer et al. (2006) noted similar increased habitat complexity in the Pascagoula River which could be a potential explanation as to why there is an increase in *H. nuchalis* after Hurricane Katrina. Species that were most affected post-storm were centrarchids and minnows (specifically *Lepomis macrochirus* and *Notropis texanus*) (Mallin and Corbett, 2006; Van Vrancken and O'Connell 2010). Species that have the ability to directly tolerate harsh storm conditions or species that can seek refuge upstream seem to be less effected after storm events. Therefore, overall fish assemblages could show drastic impact from storms with destruction to habitats in upstream reaches where species could not access refuge habitats.

Conditions in the Pascagoula River after Hurricane Katrina could have been better suited for *H. nuchalis* than *C. venusta*. Another potential explanation to the observed abundance changes following Hurricane Katrina could be attributed to food availability. *Hybognathus nuchalis* primarily feeds on decaying plant material, shed “skins” from larval insects, and fungal material (Whitaker, 1977), while *C. venusta* is primarily a drift feeder, consuming aquatic insect larvae, small seeds, and terrestrial insects (Hambrick and Hibbs, 1977; Ross, 2001). The immediate effects of Hurricane Katrina could have increased resource accessibility of detritus-based foods associated with the defoliation of riparian habitats and concentrated leaf litter that is most suitable for *H. nuchalis*.

Other studies show Cyprinid abundances changed dramatically following a hurricane. *Notropis texanus* (weed shiner) have been documented to have decreased in abundance after a hurricane event. Loss of *N. texanus* in areas directly affected by the disturbance could have been due to loss of habitat refuge after the storm (Dolloff et al., 1994) leaving the cyprinid species to be trapped in an area between decreased dissolved oxygen concentrations upstream and increase in salinity levels from storm surge in downstream reaches (Van Vrancken and O’Connell, 2010). In the case of decreasing abundances of *C. venusta* after Hurricane Katrina, the shiner could have been unable to seek refuge and therefore was not able to tolerate the conditions of the Pascagoula River. The lower DO tolerance of *C. venusta* could have hindered the species ability to withstand the effects of the storm while *H. nuchalis* could have been able to seek refuge in neighboring tributaries. In southeastern Louisiana systems, DO concentrations greater than 2.5 mg/L were favored for most freshwater fish species (Justus et al., 2014). The success of fishes to DO concentrations less than 2 mg/L were due to respiratory adaptations (ASR

or storing atmospheric air) that allowed fishes to have a higher competitive advantage. Success of these species after hypoxic events could have been attributed to reduction in competition or predation rather than lack of survivorship solely from DO concentrations. Comparing the differences in survival of species to physiological tolerances can therefore explain whether species of similar habitats share similar tolerances or not and provide insight into the niche dynamics of these fishes.

Factors influencing the distributions of fish species within stream systems are complex. Fish abundances and assemblages are driven by physiochemical tolerances to the abiotic environment (Gee et al., 1978; Scarabotti et al., 2011; Matthews and Marsh-Matthews, 2017). Understanding DO fluctuations are an important part of water-quality assessments and monitoring because oxygen influences almost all chemical and biological processes within aquatic systems. The similarities between experimental manipulations of DO tolerances and the change in relative abundances of these two fish species shown in the Pascagoula River suggests physiochemical tolerances, such as hypoxic conditions that can result after disturbances, are a major influence on fish community structure, a relationship that has been widely documented (Matthews, 1987). Therefore, increasing our understanding can only improve our knowledge on the dynamics of freshwater fish communities. With the ongoing effects of climate change, future studies should look into whether the relative abundances of species could be shifted as abiotic conditions may be altered.

2.5 REFERENCES

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2.6 Tables

Table 2.1 *Results from ANCOVA with trial as a blocked factor and size as a covariate of DO tolerance of H. nuchalis and C. venusta*

Source of Variation	Df	Sum of Squares	Mean Squares	F ratio	p
Species	1	7.468	7.468	187.514	<0.0001
Size	1	0.009	0.009	0.238	0.626
Trial	3	0.919	0.306	7.690	<0.0001
Species * Size	1	0.090	0.090	2.266	0.133
Residuals	430	16.329	0.040		

Table 2.2 *Results from ANOVA with trial as a blocked factor of LC50 of H. nuchalis and C. venusta*

Source of Variation	Df	Sum of Squares	Mean Squares	F ratio	p
Species	1	0.3760	0.3760	26.032	<0.0001
Trial	3	0.0727	0.0242	1.678	0.217
Species * Trial	2	0.0234	0.0117	0.810	0.464
Residuals	14	0.2022	0.0144		

Table 2.3 Results from ANOVA with trial as a blocked factor of ASR of *H. nuchalis* and

C. venusta

Source of Variation	Df	Sum of Squares	Mean Squares	F ratio	p
Species	1	5.962	5.962	400.962	<0.0001
Trial	3	0.011	0.004	0.244	0.864
Species * Trial	2	0.005	0.002	0.164	0.851
Residuals	14	0.208	0.015		

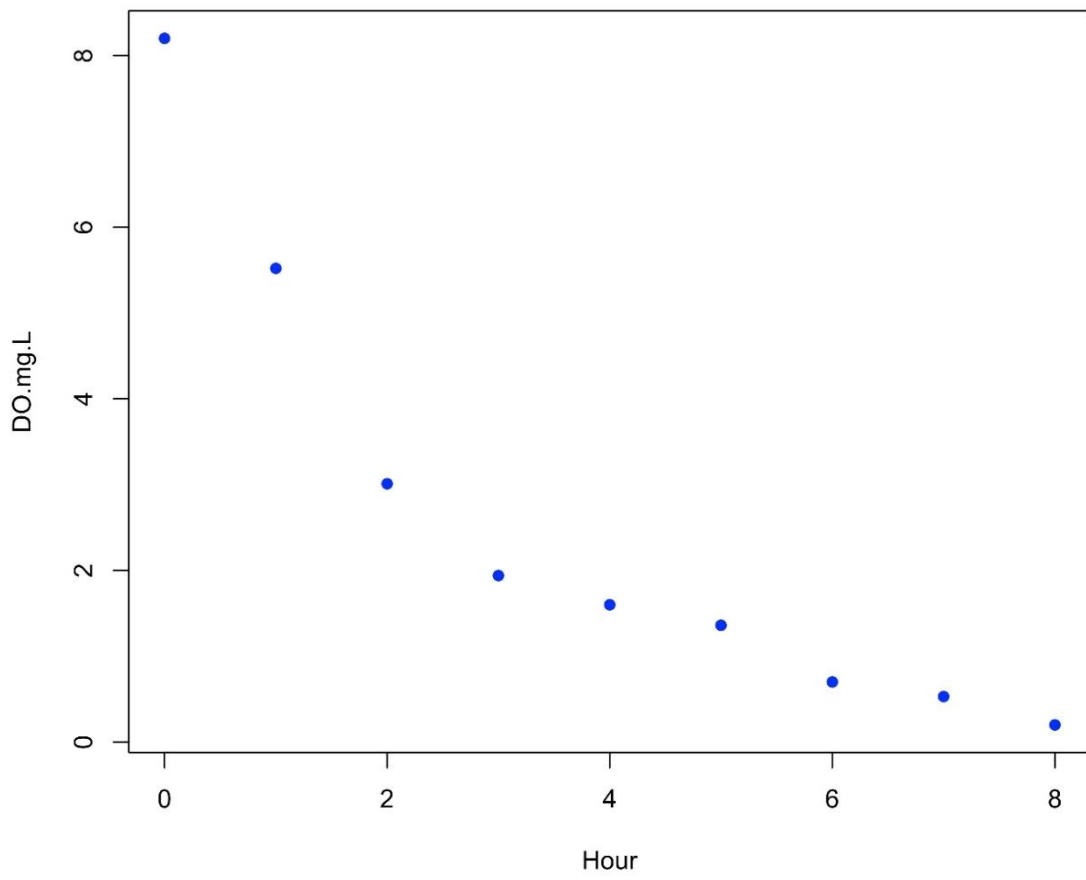


Figure 2.1 *Average rate of DO decline in experimental tanks*

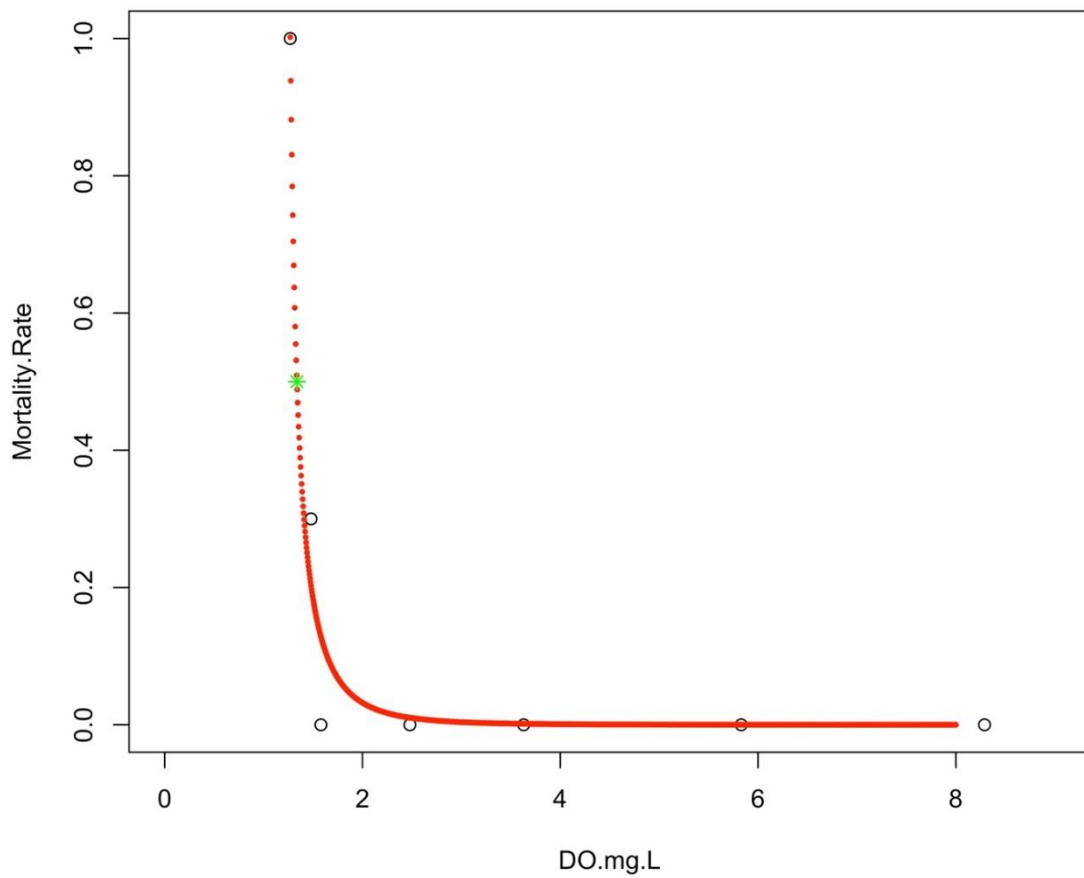


Figure 2.2 A graph showing the relationship between DO mg/L and mortality rate for one tank of *C. venusta*

Note: a 2-parameter least squares regression was then used to fit a line to predict the DO mg/L at 50% mortality (LC50). The green star indicates the predicted LC50 value from the described model.

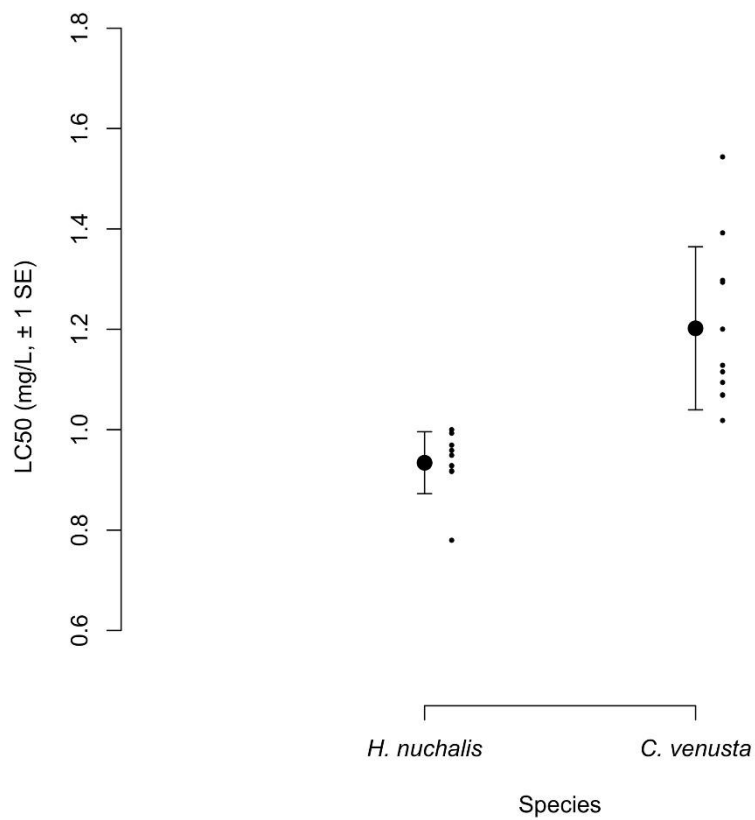
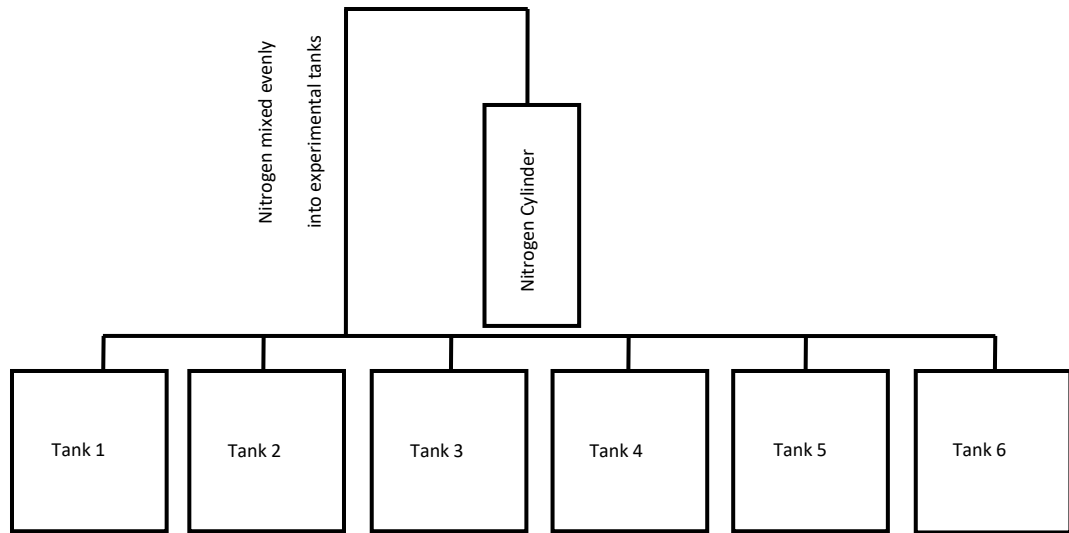


Figure 2.3 Mean LC50 of *H. nuchalis* and *C. venusta*

Note: Smaller points represent the LC50 values for each tank (*C. venusta* n = 12; *H. nuchalis* n = 10).



Scheme 2.1 *Schematic design illustrating the experimental set up.*

APPENDIX A – IACUC Approval Letter



**THE UNIVERSITY OF
SOUTHERN MISSISSIPPI**

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

118 College Drive #5116 | Hattiesburg, MS 39406-0001
Phone: 601.266.5997 | Fax: 601.266.4377 | iacuc@usm.edu | www.usm.edu/iacuc

NOTICE OF COMMITTEE ACTION

The proposal noted below was reviewed and approved by The University of Southern Mississippi Institutional Animal Care and Use Committee (IACUC) in accordance with regulations by the United States Department of Agriculture and the Public Health Service Office of Laboratory Animal Welfare. The project expiration date is noted below. If for some reason the project is not completed by the end of the approval period, your protocol must be reactivated (a new protocol must be submitted and approved) before further work involving the use of animals can be done.

Any significant changes should be brought to the attention of the committee at the earliest possible time. If you should have any questions, please contact me.

PROTOCOL NUMBER: 18121303
PROJECT TITLE: Alternative Stable States Driving Changes in Fish Assemblages in the Pascagoula River
PROPOSED PROJECT DATES: 01/2019 – 09/2020
PROJECT TYPE: New Protocol
PRINCIPAL INVESTIGATOR(S): Jake Schaefer
DEPARTMENT: School of Biological, Environmental, and Earth Sciences
FUNDING AGENCY/SPONSOR: N/A
IACUC COMMITTEE ACTION: Designated Review Approval
PROTOCOL EXPIRATION DATE: September 30, 2020

Samuel V. Bruton, PhD
Director of the Office of Research Integrity

January 16, 2019

Date