

Spring 5-11-2023

## **Freckled Darter (*Percina lenticula*) And Other Fishes' Usage Of Wood Pile Habitat**

Noah Daun

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FRECKLED DARTER (*PERCINA LENTICULA*) AND OTHER FISHES' USAGE OF  
WOOD PILE HABITAT

by

Noah James Daun

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

Approved by:

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May 2023

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## ABSTRACT

Effectively sampling habitat with the proper techniques is essential for our understanding of the animals that use the habitat, both for individual species and entire species assemblages. *Percina lenticula* is a species of special concern in the state of Mississippi due to low numbers of historical records. This species is known to inhabit wood pile habitat in rivers, a habitat that has been under-sampled historically due to inefficiency with most commonly used gear. Seines are typically not effective at sampling wood piles, resulting in sampling bias underestimating the number of *P. lenticula* and potentially other species utilizing such habitats. This study used electrofishing techniques to sample wood piles near historical records of *P. lenticula* in the Pascagoula and Pearl River drainages to assess the status of the species and environmental predictors and species associations. We assessed wood pile size and complexity for differences in fish abundance, species diversity and evenness. We also assessed differences in overall fish species assemblages in these habitats due to variations in environmental factors, both in each wood pile and at the sites. We caught 21 *P. lenticula*, all from the Pascagoula drainage. We found *Etheostoma histrio* and *Percina sciera* to be positively associated with *P. lenticula*. Neither wood pile size nor complexity significantly influenced fish abundance, species diversity or evenness. We found water depth and velocity to drive species assemblage variation in the woody structure, and site size to drive assemblage variation of the sites.

## ACKNOWLEDGMENTS

I would like to thank my advisor, Dr. Jake Schaefer, for the opportunity to work in his lab and his guidance throughout my time at Southern Mississippi. I would also like to thank Dr. Schaefer for always making time in his busy schedule to review my writing and save me from my many tussles with R. Thank you my committee members, Dr. Kaitlin Baudier and Matthew Wagner, for their advice and support throughout my project. Thank you to the other members of the lab, Matthew Aiken, Bailey Coomes, Malia Davidson, Langston Haden, and Loren Stearman for their assistance with the field work, data collection, and equipment repairs for this project. I would like to thank Jacob Zona, Dawn Thompson, Sean Weaver, Raven Ferguson, Samantha Boyd, Gabby Berry, Matthew Wagner, and Robbie Ellwanger for their help with field work. A special thanks to Dr. Mike Davis for taking great care of the generator used in the field work for this project. Finally, I would like to thank an anonymous farmer who saved the field truck when I got it stuck in loose sand on the bank of the Bogue Chitto River.

## DEDICATION

To my parents, who began my fascination with science and who have continually supported my passion and obsession with fish.

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## LIST OF ABBREVIATIONS

<i>AIC</i>	<i>Akaike Information Criterion</i>
<i>ANOVA</i>	<i>Analysis of Variance</i>
<i>cm</i>	<i>Canonical Correspondence Analysis</i>
<i>df</i>	<i>Centimeter</i>
<i>ETS</i>	<i>Electrofishing Systems LLC</i>
<i>g</i>	<i>Gram</i>
<i>GPS</i>	<i>Global Positioning System</i>
<i>IACUC</i>	<i>Institutional Animal Care And Use Committee</i>
<i>m</i>	<i>Meter</i>
<i>mm</i>	<i>Millimeter</i>
<i>NMDS</i>	<i>Non-metric Multidimensional Scaling</i>
<i>PC</i>	<i>Principle Component</i>
<i>PCA</i>	<i>Principle Component Analysis</i>
<i>s</i>	<i>Second</i>
<i>SL</i>	<i>Standard Length</i>
<i>SD</i>	<i>Standard Deviation</i>
<i>USM</i>	<i>University of Southern Mississippi</i>
<i>VIF</i>	<i>Variance Inflation Factors</i>

CHAPTER I - *Percina lenticula* (Freckled Darter) usage of woody structure in the  
Pascagoula and Pearl River Drainages

**1.1 Introduction**

Our ability to quantify species presence and abundance is essential to compiling datasets needed to study and manage ecosystems and resident biodiversity. The detection frequency of a species can vary due to sampling techniques and their efficacy in specific habitats, as sampling techniques are inherently biased (Dunn & Paukert, 2020). Using an ineffective technique for a particular species can result in a poor understanding of that species' status (Pregler et al., 2015). A species that prefers a habitat that is not frequently or effectively sampled may result in a knowledge gap for that species and the broader assemblage. Such species may be classified as data deficient and require more information on their population sizes and trends to be effectively managed (Morais et al. 2013). Data deficient species are typically at greater risk of extinction than those with quality data (Bland et al., 2014, Howard & Bickford, 2014). Challenges arise when making management decisions for these species (Astles et al., 2009) as it is unknown if the species is rare, or rarely encountered by humans. It can also be difficult to assess longer-term population trends as non-targeted surveys or surveys with inappropriate gears may not accurately assess the presence and/or abundance of a species; therefore, obtaining accurate occurrence and abundance data for such species is vital to proper management and ecosystem assessment.

Darters (subfamily: Etheostomatinae) were once coined the “hummingbirds of our freshwater fishes” by Forbes (1880) due to their small size, vibrant colors, and movement

pattern consisting of short bursts. Darters play a vital role in the incredible ichthyological diversity found in the southeastern United States. Darters are a group of over 200 species in the family Percidae that are second to only minnows (family: Leucisidae) in number of species among North American freshwater fish (Ross 2001). The southeastern United States is a hotspot of biodiversity, and fish make up a large portion of that diversity (Jenkins et al. 2018). Understanding the biology of each darter species is imperative to maintaining the remarkable diversity found in the southeastern United States.

*Percina lenticula* (Freckled Darter) was described by Richards and Knapp (1964) after its initial discovery in 1950. *Percina lenticula* is the largest species of darter (Ross 2001), reaching sizes (168 mm) over 4.5 times larger than that of the smallest darter species (Page & Burr 1979, Douglas 1968). It is a relatively understudied species, as there has been little information published about the species (Keuhne & Barbour 2014). Originally described only from the Mobile River drainage, it was later found to also inhabit the Pearl and Pascagoula River drainages as well (Suttkus & Ramsey, 1967). The range of *P. lenticula* includes areas in Georgia, Alabama, Mississippi, Tennessee, and Louisiana (Fig. 1.1).

*Percina lenticula* is a species typically associated with woody structure in areas of fast-moving and cascading water (Ross 2001, Douglas 1974). The species is also known to inhabit the downstream side of fallen trees and boulders, as well as deep potholes in bedrock (Boschung & Mayden 2004). Adults seem to prefer the midstream channel (Suttkus & Ramsey 1967), while juveniles may inhabit vegetated areas away from the main channel flow (Keuhne & Barbour 2014). The species is more common in the middle and lower reaches of the rivers it inhabits and is rare or absent at upstream sites

(Rakocinski 1968). Ross (2001) collected the species at water depths of 1.0 – 1.5m. Suttkus & Ramsey (1967) report collecting *P. lenticula* from the deepest rapids with the swiftest flows in the Leaf River, and they note an absence of *P. lenticula* in their samples in high water conditions during which such areas are not wadable.

The putative habitat of *P. lenticula* has been historically under-sampled due to the difficulty it presents for traditional sampling techniques, specifically seining. Seining tends to be less effective in deeper, swift-moving water and woody structure that entangles a seine (Kuehne & Barbour 2014). However, there are some records of the species being sampled using alternate techniques. Hubbard et al. (1991) caught *P. lenticula* in four of 77 sites in a survey on the Sucarnoochie River (Mobile River basin, Alabama & Mississippi) system via fine-meshed hoopnets. Douglas (1968) reported the capture of six *P. lenticula* from the Bogue Chitto River (Pearl River basin, Louisiana) via electrofishing and fine-meshed hoopnets. Schaefer et al. (2006) captured five *P. lenticula* specimens from the Pascagoula River via boat electrofishing while targeting juvenile *Alosa alabamae*. This noted difficulty in effectively sampling the preferred habitat of the species has likely resulted in low detection for the species and the number of *P. lenticula* in historical samples may not accurately represent the presence and/or abundance of the species. The status of the species may have been historically underestimated due to the low detection with traditional collection methods.

The scarcity of historical records can be seen when examining the records of Percina species in the Pascagoula and Pearl River drainages. *Percina lenticula* comprise the fewest records by abundance of the seven Percina species found in these drainages, making up less than 1% of the overall *Percina* records (1881-2016, Accessed through the

Fishnet2 Portal, [www.fishnet2.org](http://www.fishnet2.org), 2023-01-19; USM ichthyology portal and the University of Southern Mississippi Ichthyology Collection Portal.) (Fig. 1.2). The closely related *Percina nigrofasciata* and *Percina sciera* make up 29% and 32% of records respectively. These two species typically inhabit areas that are more suitable for surveying with seines. Ross et al. (1987) conducted a seven-year survey of Black Creek, a tributary to the Pascagoula, in which they used seines as their capture method and caught 821 *P. nigrofasciata*, 62 *P. sciera*, and zero *P. lenticula*. There are five historical records of *P. lenticula* from Black Creek, and the lack of detection in Ross' study exemplifies the potential for seining to be an ineffective technique to capture *P. lenticula*. Throughout its range *P. lenticula* is considered as vulnerable (G3) by NatureServe and Threatened by the American Fisheries Society (Warren et al. 2000). However, it is not listed under the U.S. Endangered Species Act. NatureServe lists the species critically imperiled (S1) in Louisiana and imperiled (S2) in Georgia, Alabama, and Mississippi (NatureServe 2022). This listing is consistent in Louisiana, as the Louisiana Department of Wildlife & Fisheries lists the species as S1, which requires five or fewer known extant populations in the state (Louisiana Department of Wildlife & Fisheries, 2022). It is listed as endangered in the state of Georgia (Georgia Biodiversity Portal 2009). The species is not listed at the state level in Alabama. *Percina lenticula* is listed as S2 in the state of Mississippi (Mississippi Natural Heritage Program, 2018) due to the lack of knowledge regarding the species' ecology and declining records in parts of the species' range within the state (Ross 2001). The species is thought to be stable throughout the Pascagoula River drainage due to many historical records, but there are relatively few records in the Pearl River drainage (Ross 2001). In the Pearl River drainage, the species is known from the

lower reaches of the mainstem Pearl River and two major tributaries, the Strong River and the Bogue Chitto River (Duplessis 2022). It is thought to have been extirpated from the mainstem Tombigbee River since the construction of the Tennessee – Tombigbee Waterway in the 1980's (Boschung, 1989; Hubbard et al., 1991) (Fig. 1.3).

Our objective in this study was to verify the persistence of *P. lenticula* populations in the Pearl River and Pascagoula River drainages; as such, we sampled the suspected preferred habitat of *P. lenticula* in areas with historical occurrences in both drainages. We also collected habitat data to assess environmental factors associated with the presence of *P. lenticula*, and we collected fish assemblage data to assess species positively or negatively associated with the presence of *P. lenticula*.

## **1.2 Methods**

The study took place in the Pascagoula and Pearl River drainages in southwestern Mississippi and southeastern Louisiana. Sites were selected at or near historical records of *P. lenticula* within the study area. Multiple discrete wood piles were surveyed at each site, with each wood pile constituting a unique data point (Fig. 1.4). Each wood pile was considered an individual habitat unit. Individual wood piles were considered distinct when there was roughly two meters of non-woody stream area between wood piles. The amount of wood piles surveyed per site was dependent upon the amount of assessable wood piles at the site.

Fish were sampled using backpack or barge electrofishing techniques. An ETS Badger 1 backpack electrofisher or an ETS barge electrofisher was used based on the depth and flow rate of a particular sampling site. The backpack electrofisher was used in



the shallower sites. Wood piles were sampled to depletion (until fish were no longer visually seen falling off the wood downstream into open water), and shocked fish were captured with either a dip net or a downstream seine. Captured fish were fixed in 10% buffered formalin, transported back to the lab for identification, and later moved to 70% ethanol for long-term preservation. They were then deposited into the USM ichthyology collection.

Habitat data was collected at five points per wood pile after fish sampling was completed. One data collection point was at each corner of the wood pile and one data collection point in the center. Corner points were determined in relation to the flow of the water at a wood pile. Water depth, benthic flow rate, and substrate size were taken at each point. Water depth and benthic flow rate were taken using a Hach FH950 Portable Velocity Meter. The dominant substrate size at each point was classified using a modified Wentworth scale. Substrate with grain diameter size from 0 to 2 mm were classified as sand, substrate with grain diameter size from 2.1 to 4 mm were classified as gravel, substrate with grain diameter from 4.1 to 250 mm were classified as cobble, and substrate with grain diameter 250mm or above were classified as boulder. Areas that do not have substrate grains present were classified as bedrock (Blair & McPherson, 1999). A Nikon Aculon Laser Rangefinder was used to estimate the dimensions of the wood pile by measuring the distance between the upper left point of the wood pile from the other four points. Additionally, the distance from the center point of the wood pile to each bank of the stream was measured and recorded using the rangefinder, as well as the latitude and longitude using a Garmin Oregon 450t handheld GPS. Aerial photographs were taken with a DJI Mavic Mini drone to further assess habitat variables of the wood piles. Due to

the varying sizes of the wood piles sampled, drone photographs were taken at varying heights to encompass the entire wood pile at the highest quality. A dip net of known length was included in the photo held roughly at the surface of the water for scale. Wood pile width, wood pile length, number of large wood pieces, number of small wood pieces, and major axis of the wood pile in relation to direction of flow was determined from the drone's photographs in ImageJ (Abramoff et al. 2004) using the size reference. Wood pieces with a length of 1 meter or greater were considered large wood pieces (Lamberti & Gregory 1996), and all other wood pieces visible from the drone photograph were considered small wood pieces.

The study took place in the Pascagoula and Pearl River drainages. While these drainages are close geographically, they have drastically different histories regarding human alteration and interaction. The Pascagoula River drainage drains an area of 21841 km<sup>2</sup> and is the largest river system in the contiguous United States to remain unaffected by human-caused fragmentation and flow regulation (Dynesius & Nilssen. 1994). The Pascagoula River drainage consists of two large tributaries: The Leaf River and the Chickasawhay River that meet to form the Pascagoula River. The Pearl River drainage drains an area of 14, 097 km<sup>2</sup> (Yang 1972) and has been heavily altered by human activity, including the construction of a dam to create Ross Barnett Reservoir, a 130 km<sup>2</sup> impoundment near Jackson, MS, and a 36 km navigation canal that runs parallel to the river near Bogalusa, LA (Yang 1972).

A logistic regression analysis in an AIC framework was used to determine the importance of various models taken from the environmental factors in predicting the presence of *P. lenticula* in wood piles, as well as a global model that includes all habitat

factors collected, and a null model. A PCA was also used with the environmental data collected to reduce the dimensions of the environmental factors in predicting the presence of *P. lenticula*. An indicator species analysis (Dufrene & Legendre, 1997) of species abundance was used to assess species occurrence with *P. lenticula*. A NMDS analysis was also used to further examine any potential correlative relationships other fish species may have with *P. lenticula*. All statistical tests in this study were done in R version 4.2.2 (R Core Team, 2022).

### 1.3 Results

A total of 119 wood piles were surveyed for this study: 82 wood piles at ten sites in the Pascagoula drainage and 37 at seven sites in the Pearl River drainage (Fig. 1.5). Wood piles per site ranged from five to 14, with a mean of 7.29. A total of 1052 fish were captured representing 47 species. Wood piles sampled had a mean benthic water velocity of 0.283 m/s, a mean depth of 52.9 cm. A total of 21 specimens of *P. lenticula* were captured during the survey. *Percina lenticula* specimens ranged from 68 mm to 162 mm SL, with a mean of 111.2 mm. Weight ranged from 3.12 g to 63.58 g, with a mean weight of 22.4 g (Table 1.1). *Percina lenticula* were found in wood piles with a mean velocity of 0.362 m/s, a mean depth of 57.3 cm. Gravel was the dominant substrate in five of the wood piles containing *P. lenticula*, with sand being the dominant substrate in four and bed rock being the dominant substrate in one.

Surveys on the Pascagoula River drainage took place in the fall of 2020 and were conducted on Red Creek, Black Creek, Talahalla Creek, the Leaf River, and the Chunky River (Table 1.2). A total of 567 fish were captured representing 37 species. Twenty-one

*P. lenticula* specimens were captured from 10 different wood piles, making it the 3<sup>rd</sup> most abundant *Percina* species (Fig. 1.2) and the 7<sup>th</sup> most abundant fish species captured.

Multiple *P. lenticula* specimens were captured at four wood piles, with a mean of 2.1 specimens captured per wood pile in which *P. lenticula* were present.

Surveys on the Pearl River drainage took place in the fall of 2021 and 2022. High water levels on the Pearl River in the fall of 2021 resulted in much of the Pearl River survey planned for that time being postponed until the fall of 2022. Surveys were conducted on Henderson Creek, the Strong River, the Bogue Chitto River, and the Pearl River (Table 1.2). A total of 485 fish were captured representing 37 species. No *P. lenticula* specimens were captured in the Pearl River drainage in this survey.

Due to delays in obtaining the drone only 77 of the 119 wood piles had aerial photographs taken (40 of 82 in the Pascagoula, all 37 in the Pearl). The AIC analysis identified drainage as the only predictive model for the presence of *P. lenticula*, with a weight of 0.969, followed by the null model (weight = 0.011) (Table 1.3). A logistic regression was then performed using only Pascagoula wood pile data due to the lack of *P. lenticula* captured in the Pearl, and only the null model is interpretable (weight = 0.299) (Table 1.4).

The first axis of the PCA lacking aerial photography factors described a substrate and stream size gradient, with high loadings of percent sand (1.63), percent gravel (-1.08), and stream width (0.89). The second described a substrate gradient, with high loadings of the PC2 axis being percent gravel (1.25), percent cobble (-0.95) and percent bedrock (-0.82). These first two axes described 23.5% and 16.2% of the variance, respectively (Fig. 1.6).

The first axis of the PCA that included aerial photography factors described a wood pile size and substrate gradient, with high loadings of wood pile width (1.21), wood pile diameter (1.21), and percent bedrock (0.90). The second axis described a substrate and wood pile size gradient, with high loadings of percent sand (1.08), large wood pieces (0.88), and wood pile length (0.76). These first two axes described 16.9% and 16.5% of the variance, respectively (Fig. 1.7).

The indicator species analysis identified *Etheostoma histrio* (Indicator value = 0.751) and *Percina sciera* (Indicator value = 0.623) as significant positive indicator species for *P. lenticula* (Table 1.5). The NMDS also supports the association between *P. lenticula* (NMDS 1 = 0.434, NMDS 2 = 0.681) and *E. histrio* (MDS 1 = 0.369, MDS 2 = 0.475) and between *P. lenticula* and *P. sciera* (NMDS 1 = 0.156, NMDS 2 = 0.384) (Fig. 1.8). *Etheostoma histrio* were captured at seven of the ten wood piles (70%) where *P. lenticula* was captured and were often in higher numbers than *P. lenticula*. There were 28 *E. histrio* specimens captured from wood piles with *P. lenticula*, with a mean of 2.8 *E. histrio* being collected at wood piles with *P. lenticula*. *Percina sciera* were captured at five of the ten wood piles (50%) that *P. lenticula* were, often in lower numbers than *P. lenticula*, as 11 *P. sciera* specimens were captured from wood piles with *P. lenticula*, with a mean of 1.1 *P. sciera* being collected at wood piles with *P. lenticula*. These analyses were only run using the assemblage data from the Pascagoula River drainage samples, as the Pearl River drainage samples were excluded due to the lack of *P. lenticula* specimens captured.

## 1.4 Discussion

Our results indicate that *P. lenticula* populations continue to persist in the Pascagoula River drainage in relatively high numbers, but our failure to capture any *P. lenticula* in the Pearl River drainage indicate they may occur in low abundances in that portion of their range, as recent collections of the species have been made in western areas of the Pearl River drainage by the Louisiana Department of Fish and Wildlife (Duplessis 2022, Maxwell 2022). The AIC analysis of the logistic regression models further reflected this, as drainage was the only predictive model for the presence of *P. lenticula*. This could indicate that there is another factor we didn't measure influencing the presence of *P. lenticula* within these habitats, the species' usage of these habitats is at least partially stochastic, or a low sample size. This study targeted historical localities with wood piles, and these narrow criteria could play a role in the predictive power of some of the models. Additionally, little is known about the movement of the species, it is possible that *P. lenticula* moves throughout many different wood piles or other unsampled habitats over time and were therefore not detected during this study. The wood piles in which we captured specimens during this survey could have been by chance if individual *P. lenticula* are moving between wood piles frequently. Further studies on the movement of the individual *P. lenticula* would be beneficial for the understanding and conservation of the species.

Results of the PCA without aerial photography show most of the wood piles with *P. lenticula* fell on the sand-gravel gradient of PC1. One outlier from this pattern occurred with a high amount of bed rock occurring at the wood pile it was captured from, and this was the smallest *P. lenticula* caught on the survey. Kuehne & Barbour (2014)

note that juvenile *P. lenticula* may utilize different habitats than adults. It was also captured from the wood pile with the smallest stream width among wood piles with *P. lenticula*. Adult *P. lenticula* have also been captured via trawl over gravel bars during their spawning season in the Leaf River. Many freshwater fish species use different habitats at different life stages, and more research is needed to better understand the life history of *P. lenticula*.

The results of the indicator species analysis and NMDS of *E. histrio* being strongly associated with *P. lenticula* are consistent with Suttkus & Ramsey's (1967) observation, as they observed a positive association between the two species. However, their observed positive association between *P. lenticula* and *Anguilla rostrata* is not supported by our indicator species analysis, as *A. rostrata* is a negative indicator species of *P. lenticula* in our data, albeit not significant (Indicator value = 0.166) This low indicator value is likely due to a low number (two) of *A. rostrata* being captured on our survey, with both being from wood piles lacking *P. lenticula*.

The significant positive association between *E. histrio* and *P. sciera* with *P. lenticula* in the Pascagoula River drainage may inform where *P. lenticula* may have once occurred in the Pearl River drainage. *Etheostoma histrio* and *P. sciera* were the first and fourth most abundant species in the Pearl River drainage of the study respectively, with at least one of the two species being captured at 31 of the 37 (83.8%) wood piles surveyed in the Pearl River Drainage and the two species co-occurring at 13 of the 37 (35.1%) wood piles. These three species differ in adult size, and there may be some resource partitioning by size when all three co-occur. Rakocinski (1986) found evidence for some partitioning of food items in the diets of *P. lenticula* and *P. sciera*, where the size of the

species correlated to the size of prey items consumed, with *P. lenticula* consuming larger sized prey items than *P. sciera*.

The lack of *P. lenticula* captured in the Pearl River drainage in this study may indicate the species population is following a similar trend as some other fish species that once lived in both the Pascagoula and Pearl River drainages. The ways in which human have altered the waterways in the Pearl River drainage has been linked to declines or extirpation of fish species (Warren et al. 2000), and examples of this can be seen in these this drainage. *Percina aurora* (Pearl Darter) is a federally threatened species that was historically found in both the Pascagoula and Pearl drainages but is now thought to be extirpated from the Pearl River (Bart & Suttkus, 1996) and only remains in the Pascagoula River. *Alosa alabamae* (Alabama Shad) is also thought to be extirpated from the Pearl River drainage where it once occupied (Gunning & Suttkus 1990, Smith et al., 2011), but persists in other parts of its range (Schaefer et al., 2006, Smith et al., 2011). Additionally, Tipton et al. (2004) found that areas of high disturbance in the Pearl River had a low abundance and diversity of darters opposed to areas of lower disturbance. Our results, as well as the overall decline in records of *P. lenticula* in the Pearl River drainage, may indicate a similar pattern may be occurring for *P. lenticula*.

Historically understudied species like *P. lenticula* highlight the need for focused studies to better understand the species and the habitats they occupy. These deep, swift, woody habitats undoubtedly play an important role in riverine ecosystems; however, the extent of their importance is unclear due to historical sampling biases. Studies that focus on capturing one historically understudied species can also increase the knowledge of other species that utilize the same habitat, such as *E. histrio* in this study. Studies such as



this one can help to combat the challenge the regulatory agencies responsible for making conservation and listing decisions face in determining if a species such as *P. lenticula* is a rare species, or a species that is rarely encountered by humans due to sampling bias. This study suggests that a species status can vary throughout the range of a species, as we captured *P. lenticula* at a majority of the sites sampled in the Pascagoula River drainage, but the species were absent from all sites sampled in the Pearl River drainage. As such, the populations may need to be managed differently, as the population in the Pearl River drainage may require more involved management strategies than that of the Pascagoula River drainage. Overall, studies focused on species that may have experienced historical sampling bias, such as this one, are needed to fill the knowledge gaps regarding the overall fish assemblage so we can more thoroughly understand the status of certain species and biodiversity of the southeastern United States as a whole.

## 1.5 Tables

Table 1.1 *Length and weight data of P. lenticula specimens captured in this study.*

Site	Wood Pile	Individual	Length (mm)	Weight (g)
PL 1	3	1	109	13.67
PL 3	6	1	134	32.02
PL 5	3	1	72	3.78
PL 5	5	1	84	5.59
PL 5	5	2	86	6.69
PL 6	2	1	68	3.12
PL 7	7	1	83	6.38
PL 7	7	2	84	5.88
PL 9	5	1	138	37.73
PL 10	7	1	111	16.34
PL 10	7	2	123	27.29
PL 10	10	1	102	14.41
PL 10	14	1	157	62
PL 10	14	2	124	27.78
PL 10	14	3	106	15.5
PL 10	14	4	89	7.77
PL 10	14	5	120	24.56
PL 10	14	6	101	12.75
PL 10	14	7	162	63.58
PL 10	14	8	153	53.03
PL 10	14	9	129	29.54

Table 1.2 *Site location, sampling gear, and data information for this study.*

Site ID	Latitude	Longitude	Water	Drainage	Gear	Date
PL 1	30.77157	-88.90861	Red Creek	Pascagoula	Barge Electrofischer	9/10/2020
PL 2	30.91958	-88.96575	Black Creek	Pascagoula	Barge Electrofischer	9/29/2020
PL 3	31.23276	-89.08414	Talahalla Creek	Pascagoula	Backpack Electrofischer	10/1/2020
PL 4	31.19028	-89.37646	Black Creek	Pascagoula	Backpack Electrofischer	10/1/2020
PL 5	31.43929	-89.30045	Leaf River	Pascagoula	Barge Electrofischer	10/6/2020
PL 6	32.25432	-88.85624	Chunky River	Pascagoula	Backpack Electrofischer	10/15/2020
PL 7	31.21552	-89.05654	Leaf River	Pascagoula	Barge Electrofischer	10/20/2020
PL 8	31.20401	-88.94996	Leaf River	Pascagoula	Barge Electrofischer	11/5/2020
PL 9	30.92306	-88.97259	Black Creek	Pascagoula	Backpack Electrofischer	11/10/2020
PL 10	31.35179	-89.28256	Leaf River	Pascagoula	Barge Electrofischer	11/18/2020
PL 11	30.75370	-89.82663	Pearl River	Pearl	Barge Electrofischer	11/9/2021
PL 12	31.97735	-89.89436	Strong River	Pearl	Backpack Electrofischer	8/2/2022
PL 13	31.89205	-89.97643	Strong River	Pearl	Backpack Electrofischer	9/30/2022
PL 14	30.63063	-89.88995	Bogue Chitto River	Pearl	Barge Electrofischer	10/6/2022
PL 15	30.82169	-90.12513	Henderson Creek	Pearl	Backpack Electrofischer	10/18/2022
PL 16	30.78436	-90.14447	Bogue Chitto River	Pearl	Barge Electrofischer	10/20/2022
PL 17	30.56633	-89.79536	Pearl River	Pearl	Barge Electrofischer	11/4/2022

Table 1.3 AIC analysis results of the logistic regression models using all wood piles with aerial photography data.

Factor	AIC	df	weight
Drainage	44.2	2	0.9633
Null	53.2	1	0.0107
Depth	54.8	2	0.0047
Velocity	55.2	2	0.0038
Wood Pile Size	55.3	2	0.0038
Stream Position	55.3	2	0.0037
Stream Width	55.3	2	0.0037
Wood Pieces + Wood Pile Size	56.5	5	0.0021
Global	56.6	3	0.002
Wood Pieces	56.6	3	0.002
Substrate	61.3	5	<0.001

Table 1.4 AIC analysis results for the logistic regression models using only Pascagoula drainage wood piles with aerial photography data.

Factor	AIC	df	weight
Null	42.1	1	0.299
Stream Position	43.9	2	0.118
Wood Pile Size	44.1	2	0.111
Stream Width	44.1	2	0.107
Depth	44.2	2	0.106
Velocity	44.2	2	0.104
Wood Pieces	44.3	3	0.099
Wood Pieces + Wood Pile Size	47.4	5	0.021
Global	47.8	4	0.017
Substrate	47.8	4	0.017

Table 1.5 Results of the indicator species analysis (Dufrene & Legendre, 1997) for *P. lenticula* in the Pascagoula River drainage.

Species	Association	Indicator Value	p value
<i>Etheostoma histrio</i>	Positive	0.7514	0.005
<i>Percina sciera</i>	Positive	0.6234	0.03
<i>Pylodictis olivaris</i>	Positive	0.4073	0.1
<i>Lepomis megalotis</i>	Negative	0.4966	0.18
<i>Noturus leptacanthus</i>	Positive	0.2802	0.345
<i>Cyprinella venusta</i>	Negative	0.5033	0.42
<i>Notropis longirostris</i>	Negative	0.3097	0.485
<i>Etheostoma lynceum</i>	Negative	0.3097	0.535
<i>Notropis texanus</i>	Negative	0.2867	0.57
<i>Micropterus punctulatus</i>	Negative	0.2867	0.585
<i>Ambloplites ariommus</i>	Negative	0.3677	0.67
<i>Lepomis macrochirus</i>	Negative	0.2867	0.67
<i>Pimphales vigilax</i>	Negative	0.2867	0.7
<i>Ammocrypta beani</i>	Negative	0.2341	0.805
<i>Hybopsis winchelli</i>	Negative	0.2341	0.82
<i>Percina nigrofasciata</i>	Negative	0.3612	0.98
<i>Noturus funebris</i>	Negative	0.2341	1
<i>Percina vigil</i>	Negative	0.2341	1
<i>Etheostoma swaini</i>	Negative	0.2239	1
<i>Noturus nocturnus</i>	Negative	0.2027	1
<i>Anguilla rostrata</i>	Negative	0.1655	1
<i>Lythrurus roseipinnis</i>	Negative	0.1655	1
<i>Notropis volucellus</i>	Negative	0.1655	1
<i>Hypentelium nigricans</i>	Negative	0.1655	1
<i>Gambusia affinis</i>	Negative	0.1655	1
<i>Lepomis miniatus</i>	Negative	0.1655	1
<i>Trinectes maculatus</i>	Negative	0.1655	1
<i>Ichthyomyzon gagei</i>	Negative	0.1170	1
<i>Macrhybopsis storeiana</i>	Negative	0.1170	1
<i>Luxilus chrysocephalus</i>	Negative	0.1170	1
<i>Moxostoma poecilurum</i>	Negative	0.1170	1
<i>Ictalurus punctatus</i>	Negative	0.1170	1
<i>Labidesthes sicculus</i>	Negative	0.1170	1
<i>Lepomis cyanellus</i>	Negative	0.1170	1
<i>Etheostoma stigmaeum</i>	Negative	0.1170	1
<i>Percina shumardi</i>	Negative	0.1170	1

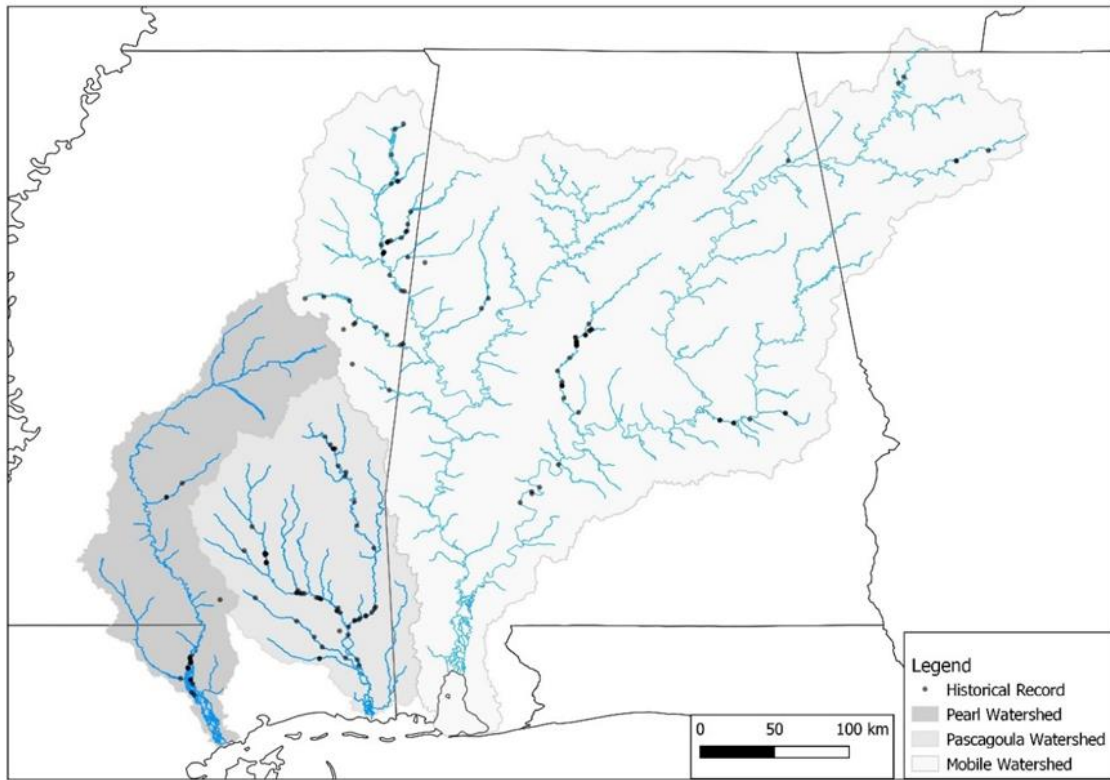


Figure 1.1 Range map of *Percina lenticula* and historic records of the species

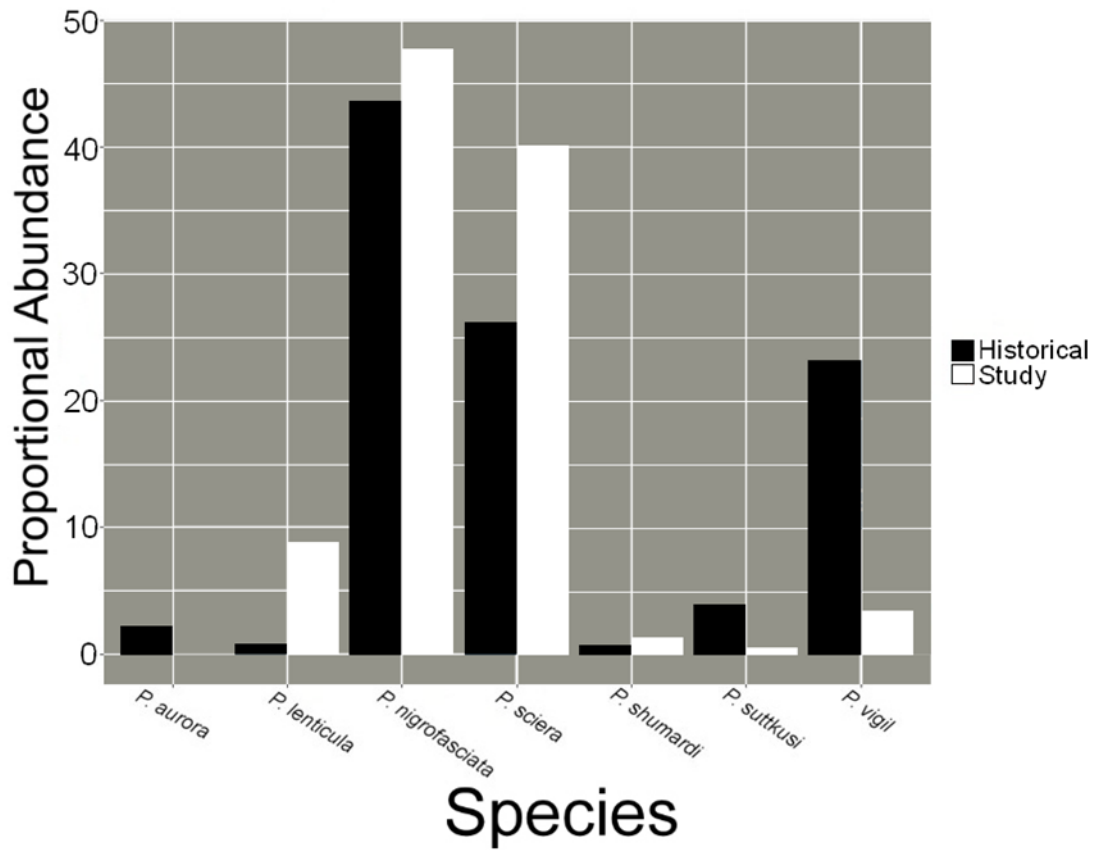


Figure 1.2 *The proportional abundance of Percina species in the study systems (1881 – 2016) and proportional abundance of Percina species from the study.*

Note: Historical fish specimen data obtained from the Mississippi Museum of Natural Science, Cornell University Museum of Vertebrates, Auburn University Museum of Natural History, Illinois Natural History Survey, Tulane University Museum of Natural History (Accessed through the Fishnet2 Portal, [www.fishnet2.org](http://www.fishnet2.org), 2023-01-19) and the University of Southern Mississippi Ichthyology Collection Portal.



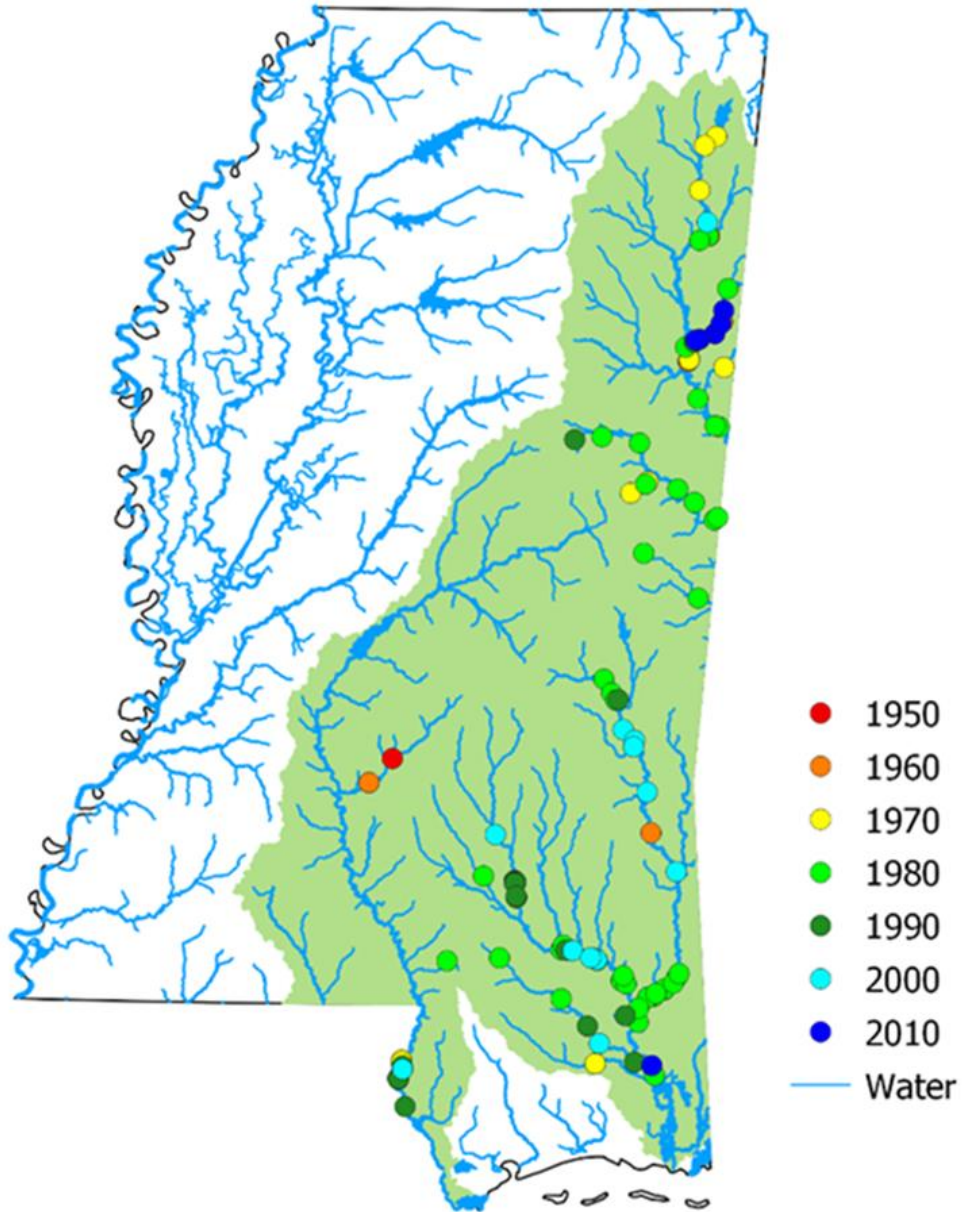


Figure 1.3 *Historical records of P. lenticula in the state of Mississippi classified by decade.*

Note. Each point represents a historical record of *P. lenticula*. Green areas indicate watersheds in which *P. lenticula* is known to occur in Mississippi.



Figure 1.4 *Example study site (PL 9) on Black Creek with five discrete wood piles and aerial photography of the wood piles.*

Note: Green points indicate wood piles where *P. lenticula* were captured, red points indicate wood piles with no *P. lenticula* captured.

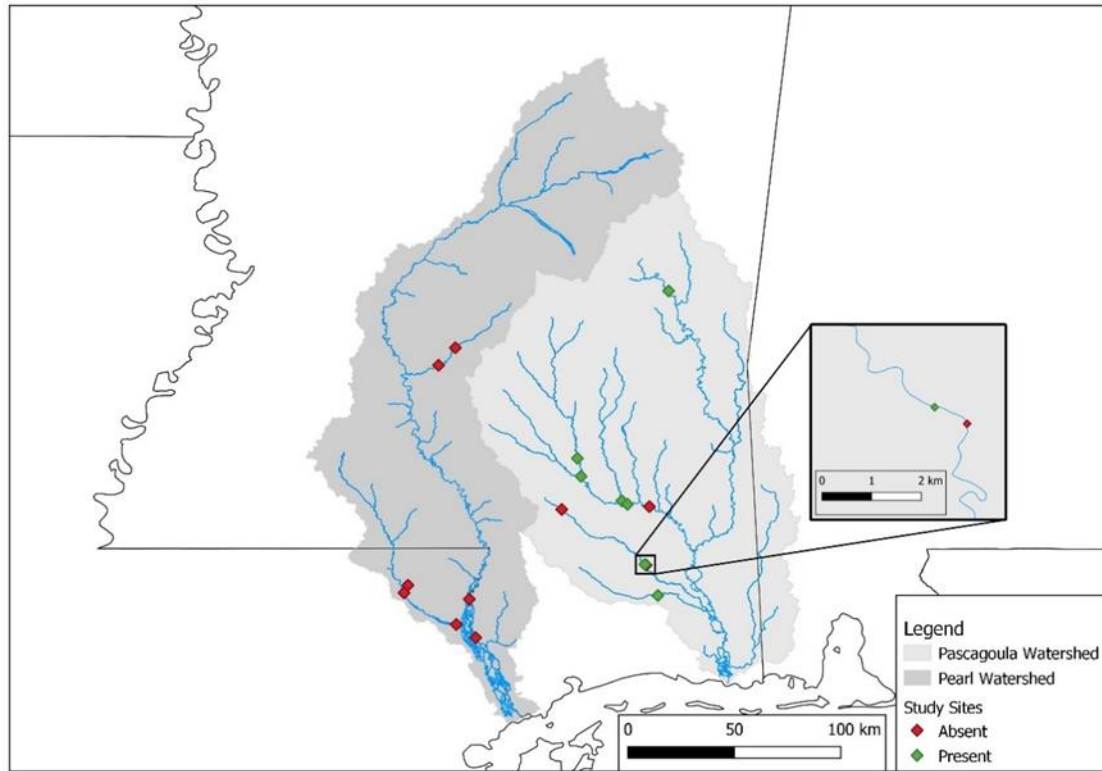


Figure 1.5 *Map of the study sites for this survey with labels of streams and river sampled.*

Note: Inset map of a portion of Black Creek is included due to proximity of two sample sites on the creek

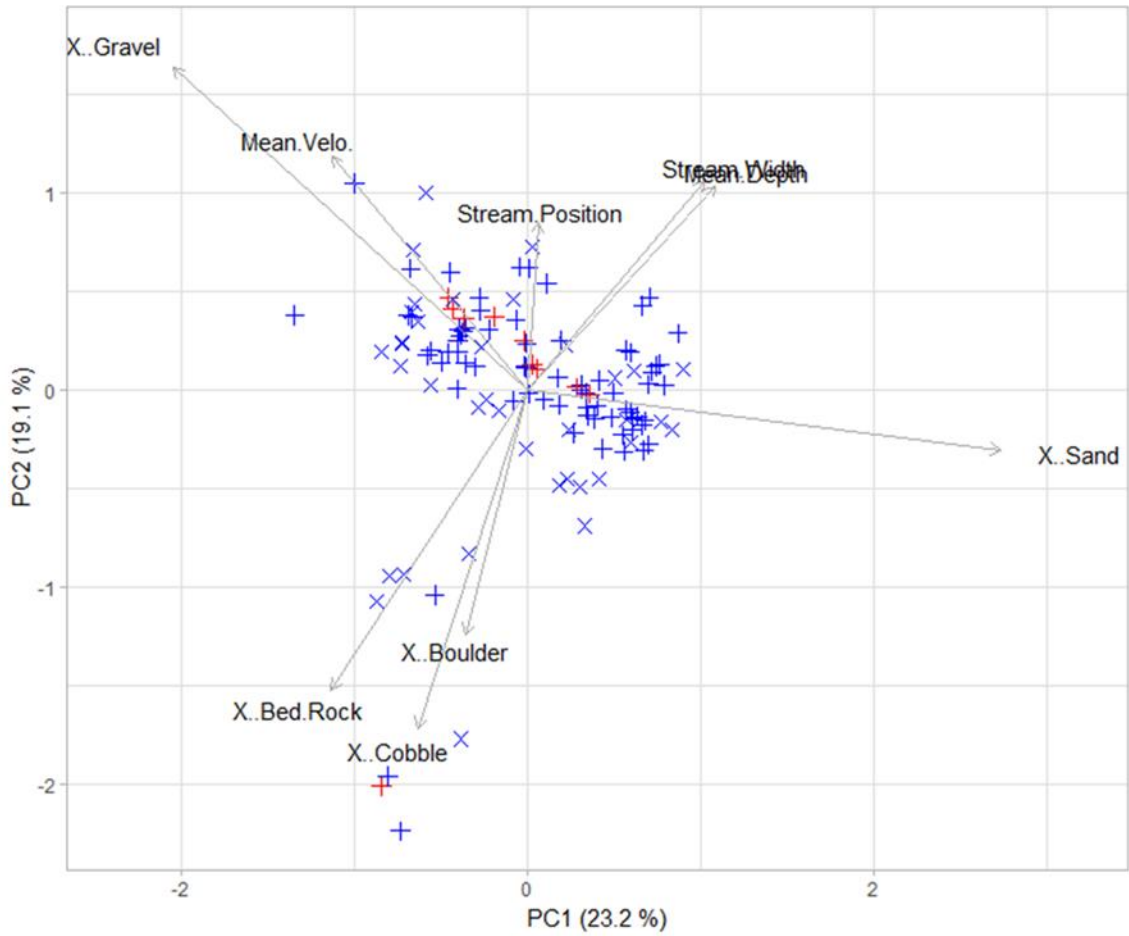


Figure 1.6 *PCA analysis of environmental factors at all sites surveyed.*

Note: Red symbols indicate wood piles where *P. lenticula* were present, blue symbols indicate wood pile where *P. lenticula* was absent. Wood piles from the Pascagoula River drainage are symbolized by + symbols, wood piles from the Pearl River drainage are symbolized by x symbols.

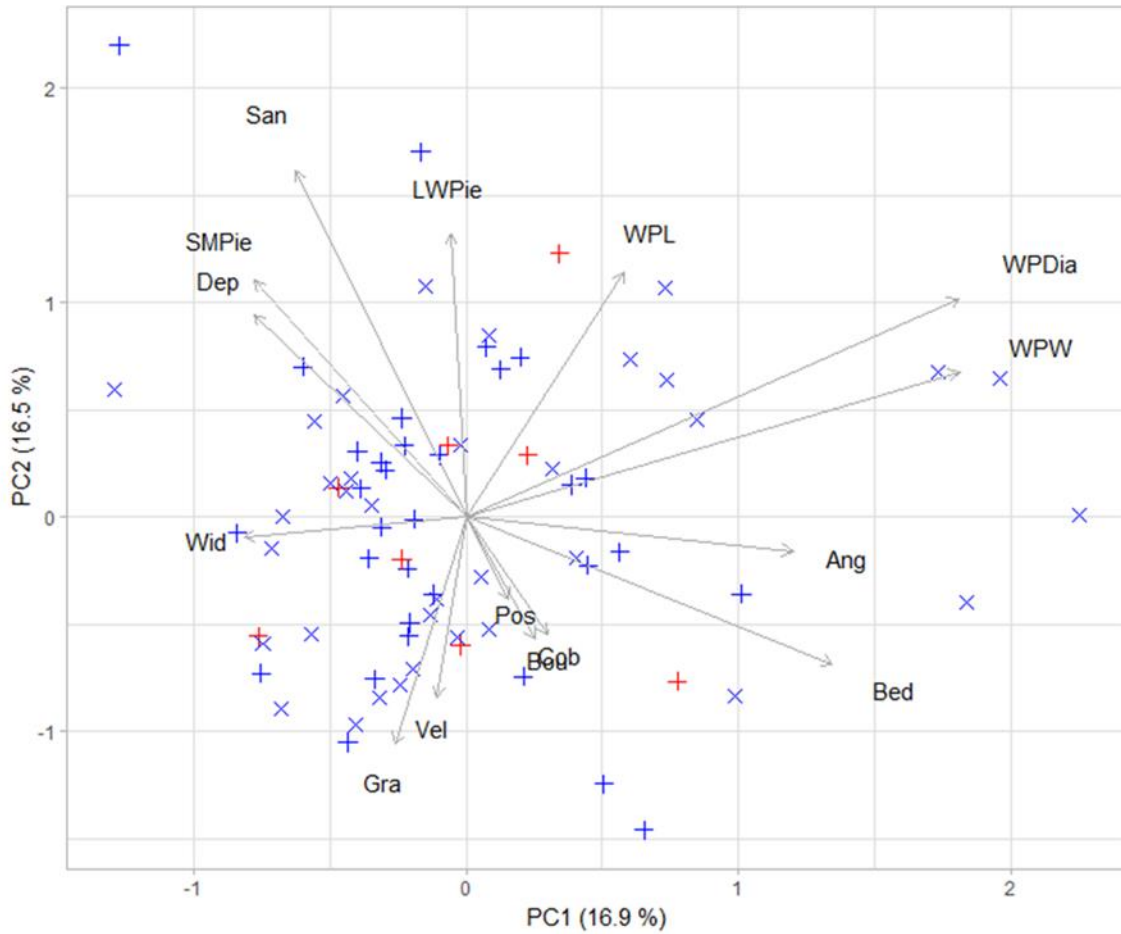


Figure 1.7 *PCA analysis of environmental factors including those gathered from aerial photography at the sites in which aerial photography was conducted on the wood piles.*

Note: Red symbols indicate wood piles where *P. lenticula* were present, blue symbols indicate wood pile where *P. lenticula* was absent. Wood piles from the Pascagoula River drainage are symbolized by + symbols, wood piles from the Pearl River drainage are symbolized by x symbols.

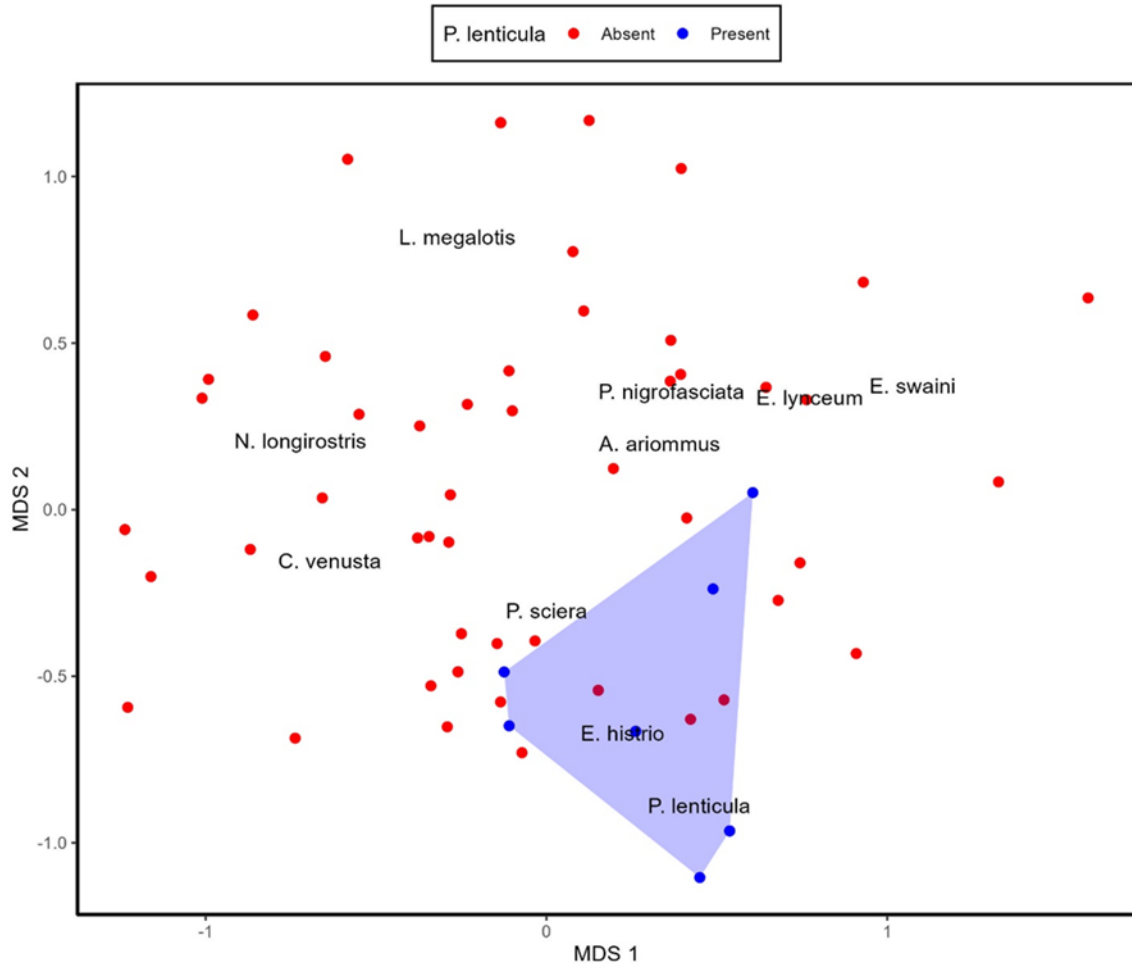


Figure 1.8 NMDS ordination of fish assemblage captured in the Pascagoula drainage with the ten most abundant species included.

Note: Wood piles with *P. lenticula* present are in blue. Wood piles without *P. lenticula* present are in red

## CHAPTER II – Fish assemblage differences in wood piles due to variations in habitat structure

### 2.1 Introduction

Understanding species diversity and assemblage dynamics at varying spatial scales is essential to understanding ecosystem processes and function. The size, structure, and complexity of habitat can influence species abundance and richness. Theory suggests larger patches of habitat can support a higher abundance of organisms and a more complex structure can support more diversity, as it provides broader niche space over which species may specialize (Menge & Sutherland, 1976, Willis et al., 2004). If such patches are isolated throughout the landscape, this complexity can give rise to metapopulations. First conceptualized by Levins (1969) when studying insects, the idea that metapopulations exist within the broader population of a species occupying patches of suitable habitat has since been observed in many other animal groups (Fretwell, 1978, Doncaster & Gustafsson, 1999, Kritzer & Sale, 2004). This concept has been studied in many marine systems, showing that an increase in habitat complexity results in higher fish diversity in seagrass (Henderson et al., 2017), artificial reef (Charbonnel et al., 2002, Gratwicke & Speight, 2005), and natural coral reef ecosystems (Roberts & Ormond, 1987, Angel & Ojeda, 2001, Darling et. al, 2017). Understanding a species' habitat use and metapopulation structure is essential when considering a species' and assemblage's distribution in space and when viewing the entirety of an ecosystem.

Riverine systems are complex and can be difficult to conceptualize due to their structure as long, thin “ribbons” of suitable habitat often isolated by terrestrial habitats



between them (Fausch et al., 2002). Despite being geographically limited, riverine systems often have a wide range of habitats that can form through various dynamic processes. These systems were often historically viewed in a framework of riffle, run, and pool complexes that form over large reaches (Yang 1971, Wohl et al., 1993). This idea may be based, in part, on the effectiveness of traditional sampling gears in these specific river habitats. Focusing on just these habitats could potentially omit smaller patches within each riffle, run, or pool that are not as effectively sampled, leaving knowledge gaps in our understanding of the system in its entirety.

Woody structures are one of the habitat patch types that are historically under-sampled or ineffectively sampled in larger systems due to the limitations of traditional gears used to sample these systems for small-bodied fish species (e.g., seines). The ways wood can influence a riverine system is vast, including the deposition of logs building meander jams and pool or bar formation (Abbe & Montgomery 1996), the condition and integrity of the riparian zone (Pusey & Arthington, 2003), and the influence on the type of substrate in a portion of a river (Martin 2001). It also can alter the flow of a river in many ways based on the size, density, and composition of the wood and its location in the river channel (Smith et al., 1993, Gurnell & Sweet, 1999, Manners et. al., 2007). The importance of wood in a riverine system can vary based on the properties of the system (Gurnell et al., 2002). A high gradient mountain stream with large substrate will likely have more variation in the available habitat for a larger amount of niche diversity to take hold throughout the river. In contrast, wood structures in a low gradient river with relatively uniform substrate provides concentrated areas of high structural habitat complexity. In such systems these wood piles act as patches of habitat for species poorly



adapted to other habitats such as open sand, and the complexity of such habitats can determine the fish community that occupies it (Gorman & Karr, 1978). This added complexity can also make fish communities more resilient to floods by providing them shelter from the temporary high velocity discharge of storm and flood events (Pearsons et al., 1992). While wood plays an important role once in a river or stream, it also plays an important role in maintaining the stability of the riverbanks as part of the riparian zone outside the channel. The health of the riparian zone and thus the integrity of the banks of a river can greatly influence the health of the river system and resident populations (Pusey & Arthington, 2003). Anthropogenic damage to the riparian zone can have profound effects on the fish assemblages in those streams. (Jones III et al., 1999, Lau et al., 2006, Zeni et al., 2019).

Woody structure in rivers and streams is important for many aquatic ecosystems and the organisms that inhabit them by creating habitat that can be used for cover (Pusey et al., 1993, Dolloff & Warren, 2003), camouflage (Angermeier & Karr, 1984), and spawning (Dolloff & Warren, 2003). The presence of wood has been shown to be positively correlated with the presence of many riverine fish species (Crook & Robertson, 1999, Warren et al. 2002), and the importance of wood is variable by species (Pettit et al., 2013). Much of the research done has focused on salmonids (Hunt 1976, Bryant 1983, Johnson et al., 2005, Moody et al., 2019) and other sportfish (Kelch et al., 1999, Lawson et al., 2011). This focus on recreationally popular species has resulted in a knowledge gap both regionally and taxonomically. Such studies fail to encompass regions and habitats that salmonids and other gamefish are not typically found in, despite these regions and habitats being ecologically diverse. The southeastern United States is a region that does

not support native salmonid populations but has the highest level of aquatic biodiversity in the country (Jenkins et al., 2015). It is also a region with a high level of riparian tree density (Lee et al., 2004), which makes the study of this habitat interaction imperative for understanding the entire ecosystem.

While relatively low in number, there have been some studies that focus on broader fish assemblage usage of woody structure. However, many of these studies look at the effects of adding wood structure to enhance or restore systems that have poor habitat complexity. Many of these concentrate on lentic systems and how the introduction of such habitat benefits anglers (Bolding et al., 2010), while fewer studies focused on native and non-game taxa in lotic systems. Such studies have found the introduction of wood into a system results in an increase in overall fish diversity and abundance, although species specific effects vary, as more benthic associated fishes such as darters (subfamily: Etheostomatinae.) and madtoms (*Noturus sp.*) had strong positive responses to introduced wood, while more open-water associated species such as minnows (family: Leuciscidae) and some sunfish (*Lepomis sp.*) had little or no response. (Warren et al., 2009, Sterling & Warren, 2018). Dugan & Rahel (2019) found that almost all species of fish in great plains rivers utilize habitat structure that is artificially added to benefit gamefish. Natural woody structure has also been found to be beneficial to fishes, as Angermeier & Karr (1984) demonstrated a positive relationship between fish abundance and increased density of woody debris in a 30.5 m section of Illinois streams. Mitchell et al. (2012) found the presence of large wood debris was associated with an increased biomass of two leuciscid species in Ozark streams. The importance of woody structure

found in these systems indicates the need to better understand fish usage of such structure in others to help illustrate the overall importance of woody structure in riverine systems.

In addition to providing cover for fish, wood also provides habitat for invertebrates that many fish species consume (Anderson et al. 1978, Benke et al. 1985, Nirulia et al., 2015). Woody structure has been shown to support a higher diversity of aquatic insects than sandy or muddy substrate (Benke et al., 1984) and supports 60% of the aquatic invertebrate biomass while only making up 4% of the total habitat structure (Benke et al., 1985). As such, increasing the amount of wood in streams has been shown to both increase the total biomass of invertebrates in a section of stream, as well as change the composition of the invertebrate guilds in that section of stream (Wallace et al., 1995). This can support an increased amount of fish diversity as it provides more opportunities for fish of differing guilds to have a reliable food source. Many studies have demonstrated that co-occurring darter species prey on different size classes of aquatic invertebrates (Wynes & Wissing, 1982, Rakocinski, 1991, Carlson & Wainwright, 2010). Therefore, a higher amount of woody structure would be likely to support varying sizes of invertebrate assemblages, allowing the opportunity for more guilds of fishes to survive, which in turn would support a higher diversity of fish species.

The difficulty sampling woody structure in larger rivers is due to the primary technique historically used, seine nets. These nets are most effective in areas of low habitat complexity due to a heavy lead line used to keep the bottom of the net down in current. The lead line gets tangled in the woody structure making fish capture difficult to impossible when this occurs (Rabeni et al., 2009). Kicking through woody structure into a seine net set immediately downstream of the wood to spook fish into the net for capture

can be an effective sampling technique, however it is typically only effective when sampling in smaller streams (Rabeni et al., 2009). Surveys that use seines as their sampling technique are likely to avoid sampling woody structure due to such difficulties, leaving these habitats unrepresented in the survey. Electrofishing is a fish sampling technique more suited towards capturing fish from woody structure. Electrofishing units are typically either mounted to a barge towed behind samplers or a backpack worn by a sampler, and they emit an electrical current in the water to stun nearby fish which can then be captured in nets. Electrofishing units typically require a greater level of effort to transport and maintain than seines, and as such have not been frequently used in surveys on larger rivers that require moving relatively far distances in the field, once again contributing to the knowledge gap regarding the species that inhabit such woody structure.

In this study we surveyed fish assemblages in wood pile structures in the Pascagoula and Pearl River drainages to determine if the size and complexity of wood piles influenced the fish evenness, diversity, and abundance. We examined these factors at a smaller scale in which each individual wood pile was considered an individual unit and at a broader scale in which each site that contained multiple wood piles in relatively close proximity was considered an individual unit. We hypothesized that larger and more complex wood piles will have higher evenness, diversity, and abundance of fish. We also tested the hypothesis that wood pile fish assemblages would respond to local wood pile habitat variability.

## 2.2 Methods

The study took place in the Pascagoula and Pearl River drainages in southern Mississippi and southeastern Louisiana. Sites were selected at or near historical records of *P. lenticula* within the study area, as the funding for this project was allocated for data collection on this understudied species that is thought to inhabit woody structure in larger waterways. Multiple discrete wood piles were surveyed at each site, with each wood pile constituting a unique data point. Individual wood piles were considered distinct when there was roughly two meters of non-woody stream area between wood piles. The amount of wood piles surveyed per site was dependent upon the amount of assessable wood piles at the site.

Fish surveys in this study were conducted using electrofishing techniques. An Electrofishing Systems LLC (ETS) Badger 1 backpack electrofisher or an ETS SDC series barge electrofisher were used based on the depth and flow rate of a particular sampling site, with the backpack electrofisher being used at shallower and slower moving sites. Wood piles were sampled to depletion (until fish were no longer visually seen falling off the wood downstream into open water), and shocked fish were captured with either a dip net or a downstream seine. Captured fish were fixed in 10% buffered formalin and transported back to the lab for identification, and later moved to ethanol for long-term preservation. They were then deposited into the University of Southern Mississippi ichthyology collection.

An aerial photograph was taken of each wood pile with a DJI Mavic Mini drone to assess habitat variables of the wood piles. Due to the varying sizes of the wood piles sampled, drone photographs were taken at varying heights to encompass the entire wood

pile at the highest image quality. A dip net of known length was included in the photo held roughly at the surface of the water for scale. Wood pile size was determined by measuring the diameter of the wood pile from the aerial images in ImageJ (Abramoff et al. 2004).

We ran a series of ANOVAs to test the effect wood pile diameter, number of wood pieces in a wood pile, and the habitat complexity index had on the total number of fish at a wood pile, the species diversity at a wood pile, and the species evenness of the fish assemblage of a wood pile. A Shannon-Wiener diversity index was used to determine the species evenness of the fish assemblage captured from each wood pile. We log transformed the data for analyses. The number of wood pieces were counted at each wood pile from the aerial photograph and were classified based on size. Wood pieces with a length of 1 meter or greater were considered large wood pieces (Lamberti & Gregory 1996), and all other wood pieces visible from the aerial photograph were considered small wood pieces. The dominant substrate type was taken at five points for each wood pile; one point in each corner and at the center point. The dominant substrate was classified using a modified Wentworth scale. Substrate with grain diameter size from 0 to 2 mm were classified as sand, substrate with grain diameter size from 2.1 to 4 mm were classified as gravel, substrate with grain diameter from 4.1 to 250 mm were classified as cobble, and substrate with grain diameter 250mm or above were classified as boulder. Points that do not have substrate grains present were classified as bedrock (Blair & McPherson, 1999). The habitat complexity index was determined by a Shannon-Wiener diversity index that included the number of large and small wood pieces and the amount of different substrate types present at the five points of each wood pile.

We ran a series of ANOVAs to test the effect of site size, number of wood pieces sampled at a site, and the habitat complexity index had on the total number of fish at a wood pile, the species diversity at a wood pile, and the species evenness of the fish assemblage of a wood pile. The size of the site was determined by the distance between the two furthest wood piles at a site, which was measured in Google Earth using the GPS coordinates taken at the sampled wood piles.

We used two CCAs to test how much assemblage variability can be explained by environmental data, one at the wood pile scale and one at the site scale. Assemblage data was transformed to proportional data for the CCA analysis. Fish species with low presence (captured at < 5 wood piles) were excluded from the CCA to reduce outlier effects. We used a stepwise modeling approach to determine the most influential environmental factors to use in the CCA. The variance inflation factors (VIF) scores of each factor were then tested for the variables to ensure a lack of collinearity. The significance of these selected variables and the significance of the CCA was tested with a permutive ANOVA. The significance of the CCA and its axes were also tested with a permutive ANOVA. All statistical tests in this study were done in R version 4.2.2 (R Core Team, 2022).

### **2.3 Results**

A total of 71 wood piles were sampled from 15 sites (Fig. 2.1): 39 wood piles from six sites in the Pascagoula River drainage and 31 wood piles from nine sites in the Pearl River drainage. A total of 588 fish were caught, representing nine families and 39 species, with 15 of the species being caught in a high enough presence to be included in

the CCA analyses (Table 2.1). A mean of 9.61 fish were caught per wood pile. The ANOVAs done on the wood piles showed no significant relationship between wood pile size, number of wood pieces, or habitat complexity and the total number of fish caught at a wood pile, species diversity or species evenness of the fish assemblage of a wood pile (Table 2.2). Likewise, the ANOVAs done on the site showed no significant relationship between site size, number of wood pieces at a site, or habitat complexity and the total number of fish caught at a wood pile, species diversity or species evenness of the fish assemblage of a site (Table 2.3).

The CCA conducted at the wood pile scale explained 7.4% of the total variance of the fish species assemblage differences between wood piles (Fig. 2.2). The CCA was found to be significant by the permutive ANOVA at  $\leq 0.01$  ( $df = 2$ ). The model selection process for the CCA resulted in the retention of two variables in the ordination: mean depth and mean velocity. The permutive ANOVA for the variables confirmed the significance of these two variables: mean velocity at  $p \leq 0.01$  ( $df = 1$ ) and mean depth at  $p \leq 0.01$  ( $df = 1$ ). The VIF scores for both variables were  $\leq 1.01$ , indicating a lack of collinearity. The first axis accounted for 3.9% of the variance and the second axis accounted for 3.4% of the variance (Fig. 2.1). The permutive ANOVA for the axes showed both to be significant (CCA1 at  $p = 0.01$ ,  $df = 1$ ,  $F$  value = 2.88; CCA2 at  $p = 0.004$ ,  $df = 1$ ,  $F$  value = 2.53).

The CCA conducted at the site scale explained 13.5% of the total variance of the fish assemblage differences between sites (Fig. 2.3). The permutive ANOVA for the CCA found it insignificant ( $p = 0.08$ ). The model selection process for the CCA resulted in the retention of one variable: site size. The permutive ANOVA for this variable



confirmed its significance at  $p \leq 0.05$ . Due to only one variable being significant the first axis accounted for the entirety of the CCA's explained variance. The permutive ANOVA for this axis found no significant axes (CCA1  $p = 0.08$ , F value = 3.38). Due to the lack of significant axes and multiple factors, other factors were included in the ordination for descriptive purposes. The selection of such factors was based on their VIF scores to reduce collinearity between the factors used, as factors with the lowest scores ( $< 1.5$ ) were kept in the CCA. Four other environmental factors were included in addition to site size: mean velocity, mean depth, mean stream width, and habitat diversity.

## **2.4 Discussion**

We failed to reject our null hypotheses, indicating no significant relationships between that wood pile size, structure, or complexity and fish abundance, species diversity or species evenness. However, some positive trends did emerge between the wood pile environmental factors and fish abundance, species diversity or species evenness. A larger sample size may have revealed more significant results similar to the trends we saw in our data. While these results show the factors that we tested did not have a direct effect on fish assemblage, it is possible that other factors we did not consider may influence the fish assemblage, or the specifics of a wood pile are not important as the presence of any wood piles. Additionally, this study was limited to wadable wood piles, and a study that utilized sampling techniques that could effectively sample wood piles at greater depth may show a different pattern.

While we were unable to determine significant relationships between fish abundance, species diversity, species evenness and wood pile structure or size, we did

find two significant factors driving assemblage structure at the wood piles: mean depth (mean = 51.91, SD = 22.37) and mean water velocity (mean = 0.29, SD = 0.27). The ordination of this data allowed us to visualize different associations the various species had along the gradients of these factors (Fig. 2.2). *Noturus nocturnus* and *Etheostoma lynceum* were associated with a higher mean velocity, while *Lepomis macrochirus* and *Etheostoma swaini* fall on the other end of the mean velocity gradient, indicating these two species are associated with lower water velocity. Stronger variation for both wood piles and species scores fell along the gradient of mean depth. *Cyprinella venusta*, and *Ambloplites ariommus* fell on the upper end of the depth gradient, with *Hypentelium nigricans*, *E. swaini*, and *Micropterus punctulatus* on the lower end. Many of the species occur near the center, suggesting that variation in depth and velocity of wood piles are not influencing the usage by many of the species, with *Lepomis megalotis* and *Noturus leptacanthus* falling closest to the center. Interestingly, the three closely related *Percina* species (subgenus: *Hadropterus*) grouped closely together, suggesting the potential for a smaller scale niche partitioning between these closely related species than we examined in this study.

We found no significant relationship between site size, wood structure or wood diversity and fish abundance, species diversity, or species evenness when testing the data at the site level. However, the data did show some of the environmental factors we tested to have positive trends on the fish abundance and assemblage metrics. The lack of significance may be a result of a relatively low sample size, and a study with several more sites could show significant results. One significant factor was found to be driving the species assemblage at the site level: site size. A larger sample size could also result in

a significant ordination and a greater number of significant factors. Our inclusion of nonsignificant environmental factors revealed some informative trends in the site level data (Fig. 2.3). *Cyprinella venusta* fell on the upper end of this axis gradient, indicating they are more likely to be found at larger sites. This is consistent with the known preferred habitats of these species, as *C. venusta* are typically found in moderate-sized to large streams (Ross 2001). *Hypentelium nigricans*, *P. nigrofasciata*, and *E. swaini* fell on the lower end, indicating they are more likely to be found at smaller sites., and these three species are noted to typically inhabit small to medium streams (Ross 2001), which is also consistent with the results of this study.

When comparing the data from both spatial levels tested, we see some similar trends are present in species associations. A group of species consisting of *H. nigricans*, *P. nigrofasciata*, and *E. swaini* clustered to a side of the gradient in both cases, suggesting these species prefer similar areas with lower water velocities and depth, at both individual wood piles and at the site level. *Micropterus punctulatus* was associated with this group at the wood piles, but not at the site level. *Noturus nocturnus* and *E. lynceum* were associated with high mean velocity areas at both scales, with *N. nocturnus* seeming to especially prefer wood piles in high water velocities. Some differences in the species associations are also present between the two levels of examination. The association between the *Percina* species in the wood piles was not as strong at the site level. *Ambloplites ariommus* and *L. megalotis* were very closely associated at the site level, but less so in the wood piles, specifically in relation to depth gradient. This suggests these two centrarchid species may coexist in close spatial proximity but could favor different wood piles based on depth. *Etheostoma histrio* and *Pylodictis olivaris*

show an opposite pattern, being closely associated at the wood pile level, but less so at the site level. These two species were near the center point of the wood pile gradients but fell out farther towards a higher mean velocity and mean depth in the site level. These species association trends at the two levels in which we examined our data can be informative when considering how various environmental factors drive the species assemblages in these habitats.

Wood piles are an important habitat component to riverine ecosystems for many fishes, our results show that the most important factor of a wood pile is merely its presence when considering the fish assemblage utilizing it. Our analyses revealed that both water depth and velocity were significant factors driving differences in fish assemblage at the wood piles, and the entire range that exists for these two factors were not fully explored due to the design of this study. As mentioned above, depth was limited by the ability of the field crew to wade at the wood pile to sample. The velocity of wood piles sampled was also biased by the selection process being influenced by historical records of *P. lenticula*. Wood piles in higher velocity areas were more likely to be sampled due to the species' suspected preference for higher velocity areas (Ross 2001) and wood piles in slower velocity areas were less likely to be sampled. Additionally, some of the fastest flowing areas were also not sampled due to safety concerns with the sampling techniques in the field. Selection at the site level was also influenced by historical records of *P. lenticula*, and a similar study with site selection independent of the historical presence of a species could return different results. Further study to expand the range sampled areas of both these significant factors would be beneficial to help

better fill the knowledge gap that exists with these important habitat components of riverine ecosystems.

## 2.5 Tables

Table 2.1 *Presence and abundance of species caught in this study.*

Species	Presence	Abundance	Abbreviation
<i>Lepisosteus oculatus</i>	1	1	-
<i>Anguilla rostrata</i>	1	1	-
<i>Cyprinella venusta</i>	19	84	Cvenu
<i>Hybognathus nuchalis</i>	2	3	-
<i>Hybopsis winchelli</i>	1	3	-
<i>Macrhybopsis storeiana</i>	2	2	-
<i>Luxilus chrysocephalus</i>	1	1	-
<i>Lythrurus roseipinnis</i>	1	1	-
<i>Notropis atherinoides</i>	2	2	-
<i>Notropis longirostris</i>	2	5	-
<i>Notropis texanus</i>	1	2	-
<i>Notropis volucellus</i>	4	5	-
<i>Pimephales vigilax</i>	4	7	-
<i>Hypentelium nigricans</i>	5	7	Hnigr
<i>Moxostoma poecilurum</i>	2	3	-
<i>Ictalurus punctatus</i>	1	1	-
<i>Noturus funebris</i>	1	1	-
<i>Noturus leptacanthus</i>	5	5	Nlept
<i>Noturus munitus</i>	1	1	-
<i>Noturus nocturnus</i>	5	5	Nnoct
<i>Noturus phaeus</i>	1	1	-
<i>Pylodictis olivaris</i>	6	7	Poliv
<i>Aphredoderus sayanus</i>	1	1	-
<i>Fundulus olivaceus</i>	1	1	-
<i>Ambloplites ariommus</i>	18	26	Aario
<i>Lepomis cyanellus</i>	1	1	-
<i>Lepomis macrochirus</i>	7	14	Lmacr
<i>Lepomis megalotis</i>	19	31	Lmega
<i>Lepomis miniatus</i>	1	1	-
<i>Micropterus punctulatus</i>	13	20	Mpunc
<i>Ammocrypta beani</i>	1	5	-
<i>Etheostoma histrio</i>	39	124	Ehist
<i>Etheostoma lynceum</i>	11	21	Elync
<i>Etheostoma stigmaeum</i>	3	3	-
<i>Etheostoma swaini</i>	10	24	Eswai
<i>Percina lenticula</i>	8	18	Plent
<i>Percina nigrofasciata</i>	29	89	Pnigr
<i>Percina sciera</i>	35	59	Pscie
<i>Percina vigil</i>	1	2	-

Table 2.2 *Results of the ANOVA tests on the environmental factors and the fish species diversity, fish species evenness, and the number of fish at a wood pile*

Environmental variable	Fish metric variable	r value	p value
Wood pile diameter	Species evenness index	0.1831	0.13
Total wood pieces	Species diversity	0.2560	0.19
Wood pile diameter	Species diversity	0.1383	0.21
Wood pile diameter	Total fish per wood pile	0.0902	0.49
Total wood pieces	Species evenness index	0.1058	0.60
Habitat complexity index	Species diversity	0.1372	0.71
Habitat complexity index	Total fish per wood pile	-0.0565	0.79
Habitat complexity index	Species evenness index	0.0749	0.94
Total wood pieces	Total fish per wood pile	0.0807	0.98

Table 2.3 *Results of the ANOVA tests on the environmental factors and the fish species diversity, fish species evenness, and the number of fish at a site*

Environmental variable	Fish metric variable	r value	p value
Habitat complexity index	Total fish per wood pile	-0.3644	0.18
Total wood pieces	Total fish per wood pile	0.3399	0.22
Total wood pieces	Species diversity	0.4245	0.24
Site Size	Total fish per wood pile	0.2818	0.29
Total wood pieces	Species evenness index	0.3290	0.29
Habitat complexity index	Species evenness index	-0.1737	0.29
Site Size	Species evenness index	-0.3254	0.34
Habitat complexity index	Species diversity	-0.3171	0.39
Site Size	Species diversity	-0.2769	0.60



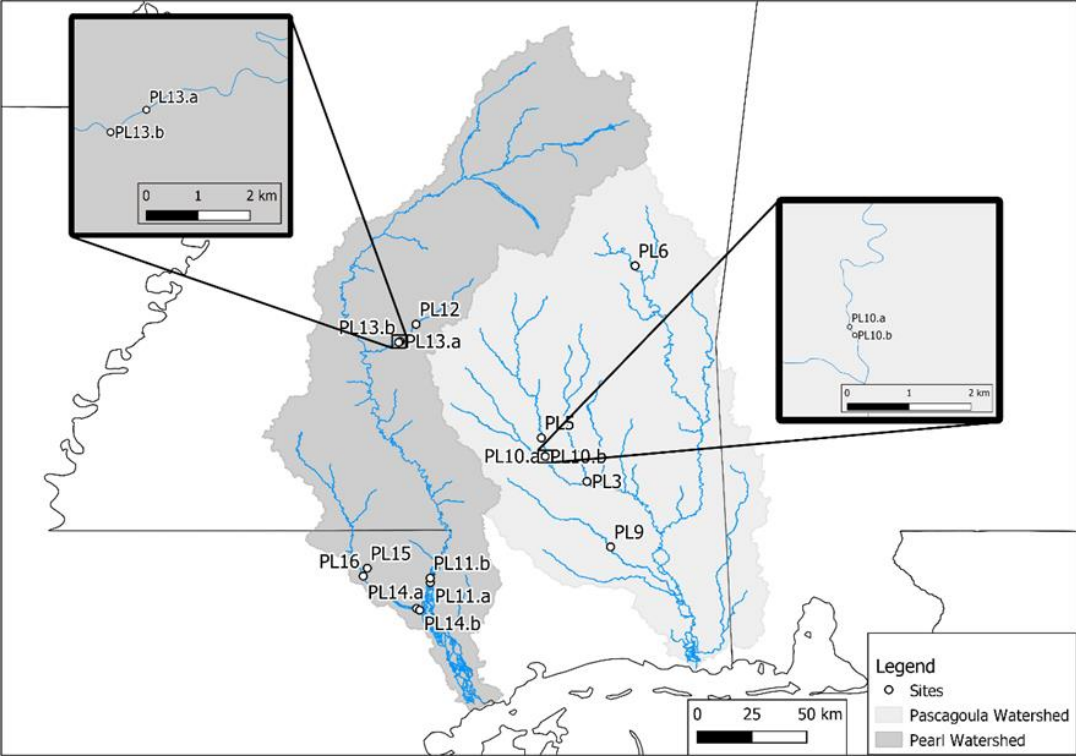


Figure 2.1 *Map of the study area and study sites*

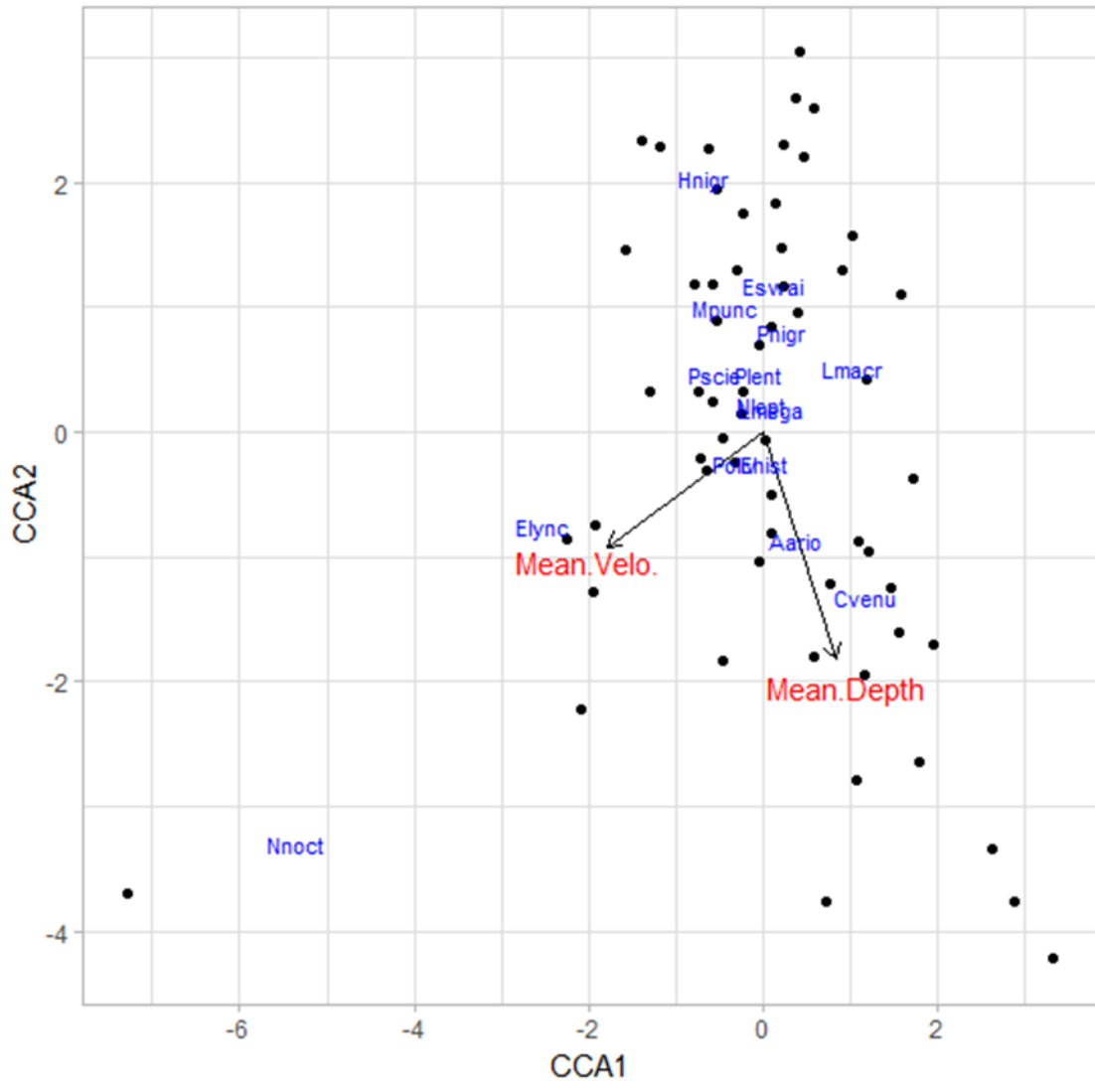


Figure 2.2 An ordination of the first two axes of the wood pile CCA

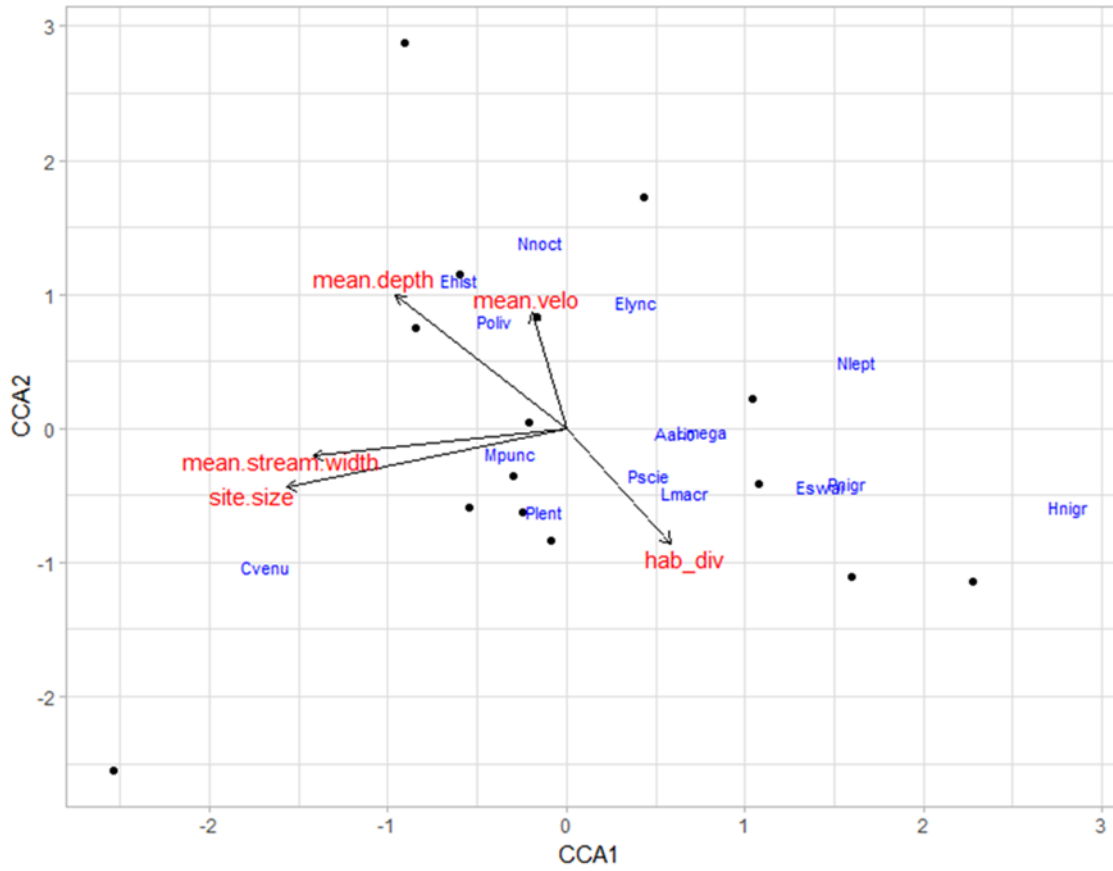


Figure 2.3 An ordination of the first two axes of the site level CCA



Table A1 (continued).

	Site	1	1	1	1	1	1	1	1	1	2	2
	Wood Pile	1	2	3	4	5	6	7	8	9	1	2
Species												
<i>Notropis atherinoides</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Notropis longirostris</i>		0	0	0	0	0	0	0	0	0	0	1
<i>Notropis texanus</i>		2	2	0	2	2	0	0	0	0	0	0
<i>Notropis volucellus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Pimephales vigilax</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Hypentelium nigricans</i>		0	2	0	0	0	0	0	0	0	0	0
<i>Moxostoma poecilurum</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Ictalurus punctatus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Noturus funebris</i>		0	0	0	1	1	2	0	0	0	1	0
<i>Noturus leptacanthus</i>		0	0	0	1	0	0	0	0	0	0	0

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Table A1 (continued).

	Site	1	1	1	1	1	1	1	1	1	2	2
	Wood Pile	1	2	3	4	5	6	7	8	9	1	2
Species												
<i>Noturus munitus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Noturus nocturnus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Noturus phaeus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Pylodictus olivaris</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Aphredoderus sayanus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Fundulus olivaceus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Gambusia affinis</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Labidesthes sicculus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Ambloplites ariommus</i>		0	0	0	0	0	0	0	0	0	0	2
<i>Lepomis cyanellus</i>		0	0	0	0	0	0	0	0	0	0	0

Table A1 (continued).

	Site	1	1	1	1	1	1	1	1	1	2	2
	Wood Pile	1	2	3	4	5	6	7	8	9	1	2
Species												
<i>Lepomis macrochirus</i>		0	0	0	0	0	0	0	0	0	0	1
<i>Lepomis megalotis</i>		0	0	0	0	0	0	0	0	0	0	2
<i>Lepomis miniatus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Micropterus punctulatus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Ammocrypta beani</i>		0	0	0	1	0	0	0	0	0	0	0
<i>Etheostoma histrio</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Etheostoma lynceum</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Etheostoma stigmaeum</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Etheostoma swaini</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Percina lenticula</i>		0	0	1	0	0	0	0	0	0	0	0





Table A1 (continued).

	Site	2	2	2	2	3	3	3	3	3	3	3
	Wood Pile	3	4	5	6	1	2	3	4	5	6	7
Species												
<i>Ichthyomyzon gagei</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Lepisosteus oculatus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Anguilla rostrata</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Cyprinella camura</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Cyprinella venusta</i>		0	0	2	0	0	2	4	0	0	0	0
<i>Hybognathus nuchalis</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Hybopsis winchelli</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Macrhybopsis storeiana</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Luxilus chrysocephalus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Lythrurus roseipinnis</i>		0	0	0	0	0	1	0	0	0	0	0



Table A1 (continued).

	Site	2	2	2	2	3	3	3	3	3	3	3
	Wood Pile	3	4	5	6	1	2	3	4	5	6	7
Species												
<i>Noturus munitus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Noturus nocturnus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Noturus phaeus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Pylodictus olivaris</i>		0	0	0	0	0	0	0	0	0	1	0
<i>Aphredoderus sayanus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Fundulus olivaceus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Gambusia affinis</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Labidesthes sicculus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Ambloplites ariommus</i>		0	0	1	0	1	0	1	0	0	0	0
<i>Lepomis cyanellus</i>		0	0	0	0	0	0	0	0	0	0	0

Table A1 (continued).

	Site	2	2	2	2	3	3	3	3	3	3	3
	Wood Pile	3	4	5	6	1	2	3	4	5	6	7
Species												
<i>Lepomis macrochirus</i>		0	1	0	0	0	0	0	0	0	0	0
<i>Lepomis megalotis</i>		0	1	0	0	0	0	2	0	0	0	0
<i>Lepomis miniatus</i>		0	0	0	1	0	1	0	0	0	0	0
<i>Micropterus punctulatus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Ammocrypta beani</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Etheostoma histrio</i>		1	0	1	0	1	0	0	1	0	1	5
<i>Etheostoma lynceum</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Etheostoma stigmaeum</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Etheostoma swaini</i>		0	0	0	0	1	0	0	0	0	0	0
<i>Percina lenticula</i>		0	0	0	0	0	0	0	0	0	1	0

Table A1 (continued).

	Site	2	2	2	2	3	3	3	3	3	3	3
	Wood Pile	3	4	5	6	1	2	3	4	5	6	7
Species												
<i>Percina nigrofasciata</i>		1	0	0	0	1	0	1	0	0	0	0
<i>Percina sciera</i>		0	0	1	1	1	0	1	0	1	0	0
<i>Percina shumardi</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Percina suttkusi</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Percina vigil</i>		0	0	1	0	0	0	0	0	0	0	0
<i>Trinectes maculatus</i>		0	0	0	0	0	0	0	0	0	0	0

Table A1 (continued).

	Site	3	4	4	4	4	4	5	5	5	5	5
	Wood Pile	8	1	2	3	4	5	1	2	3	4	5
Species												
<i>Ichthyomyzon gagei</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Lepisosteus oculatus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Anguilla rostrata</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Cyprinella camura</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Cyprinella venusta</i>		0	0	0	0	0	0	2	0	6	3	2
<i>Hybognathus nuchalis</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Hybopsis winchelli</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Macrhybopsis storeiana</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Luxilus chrysocephalus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Lythrurus roseipinnis</i>		0	0	0	0	0	0	0	0	0	0	0

Table A1 (continued).

	Site	3	4	4	4	4	4	5	5	5	5	5
	Wood Pile	8	1	2	3	4	5	1	2	3	4	5
Species												
<i>Notropis atherinoides</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Notropis longirostris</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Notropis texanus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Notropis volucellus</i>		0	0	0	0	0	0	0	0	0	1	0
<i>Pimephales vigilax</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Hypentelium nigricans</i>		0	0	1	0	0	0	0	0	0	0	0
<i>Moxostoma poecilurum</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Ictalurus punctatus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Noturus funebris</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Noturus leptacanthus</i>		0	0	0	0	1	0	0	0	0	0	0

Table A1 (continued).

	Site	3	4	4	4	4	4	5	5	5	5	5
	Wood Pile	8	1	2	3	4	5	1	2	3	4	5
Species												
<i>Noturus munitus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Noturus nocturnus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Noturus phaeus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Pylodictus olivaris</i>		0	0	0	0	0	0	0	0	0	0	1
<i>Aphredoderus sayanus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Fundulus olivaceus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Gambusia affinis</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Labidesthes sicculus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Ambloplites ariommus</i>		0	1	0	0	0	1	1	0	1	0	0
<i>Lepomis cyanellus</i>		0	0	0	0	0	0	0	0	0	0	0



Table A1 (continued).

	Site	3	4	4	4	4	4	5	5	5	5	5
	Wood Pile	8	1	2	3	4	5	1	2	3	4	5
Species												
<i>Lepomis macrochirus</i>		1	0	0	0	0	0	0	0	0	0	0
<i>Lepomis megalotis</i>		1	0	0	0	0	0	2	0	0	0	0
<i>Lepomis miniatus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Micropterus punctulatus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Ammocrypta beani</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Etheostoma histrio</i>		0	0	0	0	0	0	0	1	0	0	7
<i>Etheostoma lynceum</i>		0	2	0	2	1	1	0	0	0	1	0
<i>Etheostoma stigmaeum</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Etheostoma swaini</i>		0	0	0	0	1	0	0	0	0	0	0
<i>Percina lenticula</i>		0	0	0	0	0	0	0	0	1	0	2

Table A1 (continued).

	Site	3	4	4	4	4	4	5	5	5	5	5
	Wood Pile	8	1	2	3	4	5	1	2	3	4	5
Species												
<i>Percina nigrofasciata</i>		0	1	0	1	0	1	1	0	0	0	0
<i>Percina sciera</i>		0	0	0	0	0	0	0	0	0	0	3
<i>Percina shumardi</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Percina suttkusi</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Percina vigil</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Trinectes maculatus</i>		0	0	0	0	0	0	0	0	0	0	0

Table A1 (continued).

	Site	5	5	5	6	6	6	6	6	6	7	7
	Wood Pile	6	7	8	1	2	3	4	5	6	1	2
Species												
<i>Ichthyomyzon gagei</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Lepisosteus oculatus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Anguilla rostrata</i>		0	0	0	0	0	0	0	1	0	0	0
<i>Cyprinella camura</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Cyprinella venusta</i>		0	3	0	0	0	0	0	0	0	0	0
<i>Hybognathus nuchalis</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Hybopsis winchelli</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Macrhybopsis storeiana</i>		0	1	0	0	0	0	0	0	0	0	0
<i>Luxilus chrysocephalus</i>		0	0	0	1	0	0	0	0	0	0	0
<i>Lythrurus roseipinnis</i>		0	0	0	0	0	0	0	0	0	0	0



Table A1 (continued).

	Site	5	5	5	6	6	6	6	6	6	7	7
	Wood Pile	6	7	8	1	2	3	4	5	6	1	2
Species												
<i>Noturus munitus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Noturus nocturnus</i>		0	0	0	0	0	1	2	1	0	0	0
<i>Noturus phaeus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Pylodictus olivaris</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Aphredoderus sayanus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Fundulus olivaceus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Gambusia affinis</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Labidesthes sicculus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Ambloplites ariommus</i>		0	0	5	0	0	3	0	0	1	0	0
<i>Lepomis cyanellus</i>		0	0	0	1	0	0	0	0	0	0	0

Table A1 (continued).

	Site	5	5	5	6	6	6	6	6	6	7	7
	Wood Pile	6	7	8	1	2	3	4	5	6	1	2
Species												
<i>Lepomis macrochirus</i>		0	0	4	0	0	0	0	0	0	0	0
<i>Lepomis megalotis</i>		0	0	0	1	0	0	0	1	3	0	0
<i>Lepomis miniatus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Micropterus punctulatus</i>		0	0	0	0	0	1	0	0	0	0	0
<i>Ammocrypta beani</i>		0	5	0	0	0	0	0	0	0	0	0
<i>Etheostoma histrio</i>		1	2	0	0	2	0	0	0	0	2	2
<i>Etheostoma lynceum</i>		0	0	0	0	0	0	0	2	0	0	0
<i>Etheostoma stigmaeum</i>		0	0	0	0	0	0	0	0	1	0	0
<i>Etheostoma swaini</i>		0	0	1	2	0	0	1	0	1	0	0
<i>Percina lenticula</i>		0	0	0	0	1	0	0	0	0	0	0









Table A1 (continued).

	Site	7	7	7	7	7	7	7	8	8	8	8
	Wood Pile	3	4	5	6	7	8	9	1	2	3	4
Species												
<i>Noturus munitus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Noturus nocturnus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Noturus phaeus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Pylodictus olivaris</i>		0	1	0	0	0	0	0	0	1	0	0
<i>Aphredoderus sayanus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Fundulus olivaceus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Gambusia affinis</i>		0	0	0	0	0	0	0	0	1	0	1
<i>Labidesthes sicculus</i>		0	0	0	0	0	0	0	0	1	0	0
<i>Ambloplites ariommus</i>		0	4	0	0	0	0	0	0	0	1	0
<i>Lepomis cyanellus</i>		0	0	0	0	0	0	0	0	0	0	0

Table A1 (continued).

	Site	7	7	7	7	7	7	7	8	8	8	8
	Wood Pile	3	4	5	6	7	8	9	1	2	3	4
Species												
<i>Lepomis macrochirus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Lepomis megalotis</i>		0	0	1	0	0	0	0	1	6	0	5
<i>Lepomis miniatus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Micropterus punctulatus</i>		0	0	0	0	0	0	0	0	0	1	0
<i>Ammocrypta beani</i>		0	0	0	0	0	0	0	0	3	0	0
<i>Etheostoma histrio</i>		2	2	4	0	2	1	1	1	0	0	0
<i>Etheostoma lynceum</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Etheostoma stigmaeum</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Etheostoma swaini</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Percina lenticula</i>		0	0	0	0	2	0	0	0	0	0	0

Table A1 (continued).

	Site	7	7	7	7	7	7	7	8	8	8	8
	Wood Pile	3	4	5	6	7	8	9	1	2	3	4
Species												
<i>Percina nigrofasciata</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Percina sciera</i>		1	0	1	0	1	0	0	1	0	0	0
<i>Percina shumardi</i>		0	0	2	0	0	0	0	0	0	0	0
<i>Percina suttkusi</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Percina vigil</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Trinectes maculatus</i>		0	0	0	0	0	0	0	0	0	1	0





Table A1 (continued).

	Site	8	8	8	8	8	8	8	8	8	9	9
	Wood Pile	5	6	7	8	9	10	11	12	13	1	2
Species												
<i>Noturus munitus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Noturus nocturnus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Noturus phaeus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Pylodictus olivaris</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Aphredoderus sayanus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Fundulus olivaceus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Gambusia affinis</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Labidesthes sicculus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Ambloplites ariommus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Lepomis cyanellus</i>		0	0	0	0	0	0	0	0	0	0	0





Table A1 (continued).

	Site	8	8	8	8	8	8	8	8	8	9	9
	Wood Pile	5	6	7	8	9	10	11	12	13	1	2
Species												
	<i>Percina nigrofasciata</i>	0	0	0	0	0	0	0	0	0	3	1
	<i>Percina sciera</i>	0	1	0	0	1	0	0	0	0	0	0
	<i>Percina shumardi</i>	0	0	0	0	0	0	0	0	0	0	0
	<i>Percina suttkusi</i>	0	0	0	0	0	0	0	0	0	0	0
78	<i>Percina vigil</i>	0	0	0	0	0	0	1	0	0	0	0
	<i>Trinectes maculatus</i>	0	0	0	0	0	0	0	1	0	0	0



Table A1 (continued).

	Site	9	9	9	10	10	10	10	10	10	10	10
	Wood Pile	3	4	5	1	2	3	4	5	6	7	8
Species												
<i>Notropis atherinoides</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Notropis longirostris</i>		0	0	0	0	0	0	1	0	0	0	0
<i>Notropis texanus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Notropis volucellus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Pimephales vigilax</i>		0	0	0	1	0	0	0	0	0	0	0
<i>Hypentelium nigricans</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Moxostoma poecilurum</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Ictalurus punctatus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Noturus funebris</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Noturus leptacanthus</i>		0	0	0	0	0	0	0	0	0	0	0

Table A1 (continued).

	Site	9	9	9	10	10	10	10	10	10	10	10
	Wood Pile	3	4	5	1	2	3	4	5	6	7	8
Species												
<i>Noturus munitus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Noturus nocturnus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Noturus phaeus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Pylodictus olivaris</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Aphredoderus sayanus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Fundulus olivaceus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Gambusia affinis</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Labidesthes sicculus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Ambloplites ariommus</i>		0	0	0	0	1	0	0	0	0	0	0
<i>Lepomis cyanellus</i>		0	0	0	0	0	0	0	0	0	0	0











Table A1 (continued).

	Site	10	10	10	10	10	10	11	11	11	11	11
	Wood Pile	9	10	11	12	13	14	1	2	5	6	7
Species												
<i>Noturus munitus</i>		0	0	0	0	0	0	1	0	0	0	0
<i>Noturus nocturnus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Noturus phaeus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Pylodictus olivaris</i>		0	0	0	0	1	0	0	0	0	0	0
<i>Aphredoderus sayanus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Fundulus olivaceus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Gambusia affinis</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Labidesthes sicculus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Ambloplites ariommus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Lepomis cyanellus</i>		0	0	0	0	0	0	0	0	0	0	0

Table A1 (continued).

	Site	10	10	10	10	10	10	11	11	11	11	11
	Wood Pile	9	10	11	12	13	14	1	2	5	6	7
Species												
<i>Lepomis macrochirus</i>		0	0	2	0	0	0	0	0	0	0	0
<i>Lepomis megalotis</i>		0	0	1	0	0	0	0	0	0	0	0
<i>Lepomis miniatus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Micropterus punctulatus</i>		0	0	0	0	1	0	0	1	3	0	0
<i>Ammocrypta beani</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Etheostoma histrio</i>		3	2	0	2	3	13	3	0	2	2	2
<i>Etheostoma lynceum</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Etheostoma stigmaeum</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Etheostoma swaini</i>		0	1	0	0	0	0	0	0	1	0	0
<i>Percina lenticula</i>		0	1	0	0	0	9	0	0	0	0	0



Table A1 (continued).

	Site	12	12	12	12	12	12	12	13	13	13	13
	Wood Pile	1	2	3	4	5	6	7	1	2	3	4
Species												
<i>Ichthyomyzon gagei</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Lepisosteus oculatus</i>		0	0	0	0	0	0	1	0	0	0	0
<i>Anguilla rostrata</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Cyprinella camura</i>		0	0	3	0	0	0	0	0	0	0	0
<i>Cyprinella venusta</i>		4	3	0	0	0	0	1	0	6	0	0
<i>Hybognathus nuchalis</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Hybopsis winchelli</i>		0	0	0	0	0	0	3	0	0	0	0
<i>Macrhybopsis storeiana</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Luxilus chrysocephalus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Lythrurus roseipinnis</i>		0	0	0	0	0	0	0	1	0	0	0

Table A1 (continued).

	Site	12	12	12	12	12	12	12	13	13	13	13
	Wood Pile	1	2	3	4	5	6	7	1	2	3	4
Species												
<i>Notropis atherinoides</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Notropis longirostris</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Notropis texanus</i>		0	0	0	0	0	0	0	3	2	0	0
<i>Notropis volucellus</i>		1	0	0	0	0	0	0	0	0	0	0
06 <i>Pimephales vigilax</i>		0	0	0	0	0	0	4	4	0	0	0
<i>Hypentelium nigricans</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Moxostoma poecilurum</i>		0	0	0	0	0	0	1	0	0	0	0
<i>Ictalurus punctatus</i>		0	0	0	0	1	0	0	0	0	0	0
<i>Noturus funebris</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Noturus leptacanthus</i>		2	1	0	0	0	1	0	1	0	0	1

Table A1 (continued).

	Site	12	12	12	12	12	12	12	13	13	13	13
	Wood Pile	1	2	3	4	5	6	7	1	2	3	4
Species												
<i>Noturus munitus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Noturus nocturnus</i>		0	0	0	0	0	0	0	3	0	0	0
<i>Noturus phaeus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Pylodictus olivaris</i>		0	1	0	0	0	0	0	0	0	0	0
<i>Aphredoderus sayanus</i>		0	0	0	0	0	0	1	0	0	0	0
<i>Fundulus olivaceus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Gambusia affinis</i>		0	0	1	0	0	0	0	0	0	0	0
<i>Labidesthes sicculus</i>		0	0	1	0	0	0	0	0	0	0	0
<i>Ambloplites ariommus</i>		1	1	0	1	0	0	0	0	1	1	0
<i>Lepomis cyanellus</i>		0	0	0	0	0	0	0	0	0	0	0

Table A1 (continued).

	Site	12	12	12	12	12	12	12	13	13	13	13
	Wood Pile	1	2	3	4	5	6	7	1	2	3	4
Species												
<i>Lepomis macrochirus</i>		0	0	0	0	0	0	1	0	0	4	0
<i>Lepomis megalotis</i>		0	1	0	1	0	1	1	1	0	5	0
<i>Lepomis miniatus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Micropterus punctulatus</i>		2	0	0	0	1	1	1	0	0	2	0
<i>Ammocrypta beani</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Etheostoma histrio</i>		1	1	0	3	0	1	0	3	3	0	8
<i>Etheostoma lynceum</i>		9	0	10	1	0	0	0	0	0	0	5
<i>Etheostoma stigmaeum</i>		0	0	0	0	0	0	0	0	0	1	0
<i>Etheostoma swaini</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Percina lenticula</i>		0	0	0	0	0	0	0	0	0	0	0

Table A1 (continued).

	Site	12	12	12	12	12	12	12	13	13	13	13
	Wood Pile	1	2	3	4	5	6	7	1	2	3	4
Species												
<i>Percina nigrofasciata</i>		0	2	4	6	0	5	1	0	0	2	1
<i>Percina sciera</i>		5	1	18	4	1	3	2	0	1	0	1
<i>Percina shumardi</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Percina suttkusi</i>		0	1	0	0	0	0	0	0	0	0	0
<i>Percina vigil</i>		0	0	2	0	0	0	0	0	0	0	0
<i>Trinectes maculatus</i>		0	0	0	0	0	0	0	0	0	0	0



Table A1 (continued).

	Site	13	13	13	14	14	14	14	14	15	15	15
	Wood Pile	5	6	7	1	2	3	4	5	1	2	3
Species												
<i>Ichthyomyzon gagei</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Lepisosteus oculatus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Anguilla rostrata</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Cyprinella camura</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Cyprinella venusta</i>		0	0	0	0	0	1	5	7	0	0	0
<i>Hybognathus nuchalis</i>		0	0	0	0	0	0	0	1	0	0	0
<i>Hybopsis winchelli</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Macrhybopsis storeiana</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Luxilus chrysocephalus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Lythrurus roseipinnis</i>		0	0	0	0	0	0	0	0	0	0	0



Table A1 (continued).

	Site	13	13	13	14	14	14	14	14	15	15	15
	Wood Pile	5	6	7	1	2	3	4	5	1	2	3
Species												
<i>Noturus munitus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Noturus nocturnus</i>		0	0	1	1	0	0	0	1	0	0	0
<i>Noturus phaeus</i>		0	0	0	0	0	0	0	0	1	0	0
<i>Pylodictus olivaris</i>		0	0	1	0	0	0	0	2	0	0	0
<i>Aphredoderus sayanus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Fundulus olivaceus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Gambusia affinis</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Labidesthes sicculus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Ambloplites ariommus</i>		1	0	2	0	0	0	0	2	1	0	0
<i>Lepomis cyanellus</i>		0	0	0	0	0	0	0	0	0	0	0

Table A1 (continued).

	Site	13	13	13	14	14	14	14	14	15	15	15
	Wood Pile	5	6	7	1	2	3	4	5	1	2	3
Species												
<i>Lepomis macrochirus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Lepomis megalotis</i>		0	1	3	0	0	1	0	2	0	0	0
<i>Lepomis miniatus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Micropterus punctulatus</i>		0	0	0	0	0	0	0	4	0	0	0
<i>Ammocrypta beani</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Etheostoma histrio</i>		0	7	3	6	2	3	14	4	0	0	1
<i>Etheostoma lynceum</i>		1	1	0	0	0	0	4	0	0	1	0
<i>Etheostoma stigmaeum</i>		0	1	0	0	0	0	0	0	0	0	0
<i>Etheostoma swaini</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Percina lenticula</i>		0	0	0	0	0	0	0	0	0	0	0



Table A1 (continued).

	Site	15	15	15	16	16	16	16	16	16	16	17
	Wood Pile	4	5	6	1	2	3	4	5	6	7	1
Species												
<i>Ichthyomyzon gagei</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Lepisosteus oculatus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Anguilla rostrata</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Cyprinella camura</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Cyprinella venusta</i>		2	0	0	0	1	0	0	0	13	0	3
<i>Hybognathus nuchalis</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Hybopsis winchelli</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Macrhybopsis storeiana</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Luxilus chrysocephalus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Lythrurus roseipinnis</i>		0	0	0	0	0	0	0	0	0	0	0

Table A1 (continued).

	Site	15	15	15	16	16	16	16	16	16	16	17
	Wood Pile	4	5	6	1	2	3	4	5	6	7	1
Species												
<i>Notropis atherinoides</i>		0	0	0	0	0	0	0	0	1	0	0
<i>Notropis longirostris</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Notropis texanus</i>		0	0	0	0	0	0	0	0	0	0	3
<i>Notropis volucellus</i>		0	0	0	0	0	0	0	0	1	0	0
<i>Pimephales vigilax</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Hypentelium nigricans</i>		2	2	1	0	0	0	0	0	1	0	0
<i>Moxostoma poecilurum</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Ictalurus punctatus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Noturus funebris</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Noturus leptacanthus</i>		0	0	1	0	0	0	0	0	0	0	0

Table A1 (continued).

	Site	15	15	15	16	16	16	16	16	16	16	17
	Wood Pile	4	5	6	1	2	3	4	5	6	7	1
Species												
<i>Noturus munitus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Noturus nocturnus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Noturus phaeus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Pylodictus olivaris</i>		0	0	0	0	1	0	0	0	0	0	0
<i>Aphredoderus sayanus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Fundulus olivaceus</i>		1	0	0	0	0	0	0	0	0	0	0
<i>Gambusia affinis</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Labidesthes sicculus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Ambloplites ariommus</i>		1	1	0	0	0	0	0	0	1	0	1
<i>Lepomis cyanellus</i>		0	0	0	0	0	0	0	0	0	0	0



Table A1 (continued).

	Site	15	15	15	16	16	16	16	16	16	16	17
	Wood Pile	4	5	6	1	2	3	4	5	6	7	1
Species												
<i>Lepomis macrochirus</i>		0	1	0	0	0	0	0	0	0	0	2
<i>Lepomis megalotis</i>		0	0	0	0	1	0	0	0	1	0	12
<i>Lepomis miniatus</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Micropterus punctulatus</i>		1	0	0	0	0	0	0	1	1	0	1
<i>Ammocrypta beani</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Etheostoma histrio</i>		0	0	0	3	4	0	1	1	0	0	0
<i>Etheostoma lynceum</i>		0	0	1	0	3	0	1	0	0	0	0
<i>Etheostoma stigmaeum</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Etheostoma swaini</i>		4	10	0	0	0	0	0	0	2	0	0
<i>Percina lenticula</i>		0	0	0	0	0	0	0	0	0	0	0

Table A1 (continued).

	Site	15	15	15	16	16	16	16	16	16	16	17
	Wood Pile	4	5	6	1	2	3	4	5	6	7	1
Species												
<i>Percina nigrofasciata</i>		5	15	6	0	4	0	0	0	1	0	0
<i>Percina sciera</i>		0	0	0	0	8	1	0	0	1	1	4
<i>Percina shumardi</i>		0	0	0	0	0	0	0	0	0	0	1
<i>Percina suttkusi</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Percina vigil</i>		0	0	0	0	0	0	0	0	0	0	0
<i>Trinectes maculatus</i>		0	0	0	0	0	0	0	0	0	0	0

APPENDIX B – 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: 21021101  
PROJECT TITLE: Survey for Pearl Darters (*Percina aurora*) in the Pearl and  
Pascagoula Basins  
PROPOSED PROJECT DATES: 03/2021 - 09/2022  
PROJECT TYPE: New Protocol  
PRINCIPAL INVESTIGATOR(S): Jake Schaefer  
DEPARTMENT: Biological Sciences  
FUNDING AGENCY/SPONSOR: N/A  
IACUC COMMITTEE ACTION: Committee Approval  
PROTOCOL EXPIRATION DATE: September 30, 2022

Samuel Bruton, PhD  
Director, Office of Research Integrity

March 3, 2021

Date

## APPENDIX C REFERENCES

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