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The Ecology and Evolution of the Freshwater Turtles of Southern Mississippi

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THE ECOLOGY AND EVOLUTION OF THE FRESHWATER TURTLES OF SOUTHERN MISSISSIPPI

by

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ABSTRACT

Turtles are one of the most threatened group of animals in existence today. The Southeastern United States is one of two global biodiversity hotspots for turtle species, including the state of Mississippi, where over 30 species can be found. However, very few studies have occurred within the state. This lack of research is even more startling given the ongoing decline, or even extirpation, of numerous turtle species across the world, due to a number of factors, including habitat degradation, and harvest for food or the pet trade.

The overarching goal of this project was to perform a species inclusive freshwater survey and document the distribution and abundances of the diverse species present here. A substantial amount of data was collected through these surveys, including morphometric measurements, genetic samples, and habitat data recorded at each trap location. These data were then used to determine if riverine habitat and surrounding land cover has any effect on turtle communities. Similarly, a state-wide population genetic study on the Spiny Softshell turtle (*Apalone spinifera)* was initiated.

The surveys performed for this study captured a total of 1,230 turtles, from 16 species. Analyses showed that land-use had no significant impact on turtle communities or species, but that habitat can be a predictor of species occurrence in some circumstances. Finally, our genetic analysis of *A. spinifera* from the Pascagoula and Pearl River drainages showed two distinct populations between the two drainages, but did not detect any intra-drainage populations structure.

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DEDICATION

To my parents, Kathy and Don Berry. I could not have made it this far without the constant reminder to go after the things that I love and care about, to do my best and try my hardest. To Kevin Bohl, thanks for sticking by my side through every step of this journey. And to all the friends I've made along the way.

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1.1 Introduction

Turtles, of the order Testudines, are a highly derived group of reptiles that are easily distinguished by their strengthened anapsid skull, the position of the limb girdles inside the ribcage, and an external bony shell covered in keratinous scutes (Zardoya & Meyer, 2001). These unique characteristics have evolved over many millennia, with one of the earliest known stem-turtle ancestors, *Pappochelys rosinae,* which more resembled a stout lizard rather than its turtle lineage, living over 240 million years ago (mya) during the middle Triassic period (Schoch & Sues, 2015). Over this great span of time, turtles were able to radiate across the globe and in modern times have diversified into 356 unique species (Rhodin et al, 2017). All extant species can be grouped into two main suborders: 1) Cryptodira (vertical-necked turtles) which arose in the late Jurassic period around 150 mya, and 2) Pleurodira (side-necked turtles), a much older group which dates back to the late Triassic period over 200 mya (Vitt & Caldwell, 2013). Turtles within the group Pleurodira are either aquatic or semi-aquatic and are restricted to the Southern hemisphere, inhabiting parts of South America, Australia, and New Guinea (Vitt & Caldwell, 2013). Found throughout the Northern hemisphere and parts of the Southern hemisphere (South America and Africa), Crpytodires occur in an array of habitats including terrestrial, freshwater, and marine (Plough et al., 2009).

Turtles are an extremely long-lived group of animals, especially when compared to the squamate reptiles (Vitt & Caldwell, 2013), and as adults they have extremely high survivorship (Galbraith & Brooks, 1987). As a whole, turtles are generally late to mature but are then able to reproduce for an extended period of time (Ernst & Lovich, 2009), with most mortality occurring in the egg or juvenile life stages (Congdon et al., 1983). This type of life history is perfectly viable for the animal in a natural environment, as adults have very few natural predators and are able to reproduce for as long as decades in some cases (Ernst & Lovich, 2009). However, due to man-made pressures (harvest, habitat destruction, etc.), this slow reproductive strategy is hindering the group's survival and has led to turtles being one of the most at-risk groups of animals in the world (Rhodin et al., 2011).

Of the 356 known extant species, 149 (42.8%) are considered threatened and 84 (24.1%) are considered endangered or critically endangered (Turtle Taxonomy Working Group, 2017). There are numerous factors leading to these startling numbers which include habitat degradation, fragmentation, and destruction, and harvest for the pet trade (Fund, T.C., 2002). However, the illegal overharvest and exploitation of wild turtles for food and traditional medicinal purposes, centered in Asia, is the number one factor leading to the swift decline in turtle populations (van Dijk et al., 2000).

The harvest of turtles for food is not a recent phenomenon, and neither is it restricted to Asia. Historically freshwater, marine, and terrestrial turtles have all been hunted for both their meat or eggs (Klemens & Thorbjarnarson, 1995). In recent history, the United States has likewise utilized a number of turtle species for their meat. Historic declines in marine turtles, such as the Green Sea turtle (*Chelonia mydas*), have been attributed to numerous factors including the theft of eggs and commercial harvesting of adults (Wyneke et al., 1998). This harvesting was halted in the U.S. when all sea turtles were designated as endangered and became federally protected (IUCN, 1996). However, the demand for turtle meat remained, leading to the overharvest of other large species like the Alligator Snapping turtle (*Macrochelys temminckii*), which was harvested throughout its range into the late 1980s (Pritchard, 1989; Sloan and Lovich, 1995). The Alligator Snapping turtle is now protected throughout its range, with the exception of Louisiana and Mississippi, and is being considered for federal protection due to its extreme range-wide decline.

Size is not the only factor that leads to overharvest. The Diamond-backed Terrapin (*Malaclemys terrapin*), which is a medium-sized estuarine species, much smaller than the Alligator Snapping turtle or a sea turtle, was exploited for close to 300 years (Carr 1952, Hay 1904; McCauley 1945), until populations were reduced to such low levels that extinction was feared (Babcock 1926; Carr 1952; De Sola 1931; Hay 1904). The species is beginning to bounce-back (Burke et al. 2000; Carr 1952; Klemens 1993), however it is likewise being considered for federal protection (CITES, 2013).

While overall harvest has lessened considerably, and most species seem to be rebounding from devastating population declines that this exploitation has caused, massive amounts of turtles are still being taken from the wild, either as a food source or to be exported. In the state of Arkansas, for example, 126,381 freshwater turtles were harvested from 2014 to 2016 (Bennett, 2018). However, this number pales in comparison to exploitation in Southeast Asia, where China is the world’s leading consumer of turtle meat and is considered a primary threat to the world’s turtle populations (Brown et al., 2011; Compton, 2000; Mali et al., 2014; van Dijk, 2000). This illegal and unsustainable trade of freshwater turtles and tortoises in Asia has been dubbed the Asian Turtle Crisis (Barzyk, et al., 2002) and has led to steep population declines (van Dijk et al., 2000b).

Illegal trade is a major issue and a severe conservation threat (Nijman & Shepher, 2014), and this crisis is not reserved for species native to Asia. Over a ten-year period, Nijman & Shepher recorded a total of 2,667 individual turtles, representing 55 species, for sale in the largest outdoor market in Thailand, Bangkok's Chatuchak weekend market (2002). The majority of these species were not native to Thailand, with 372 individuals from 16 North American species, such as *Macrochelys temminickii*, *Malaclemmys terrapin*, and *Sternotherus odoratus,* recorded (Nijman & Shepher, 2014).

With the collapse of Asian turtle populations, international importation has begun to increase dramatically (Haitao et al., 2008). The bulk of turtles exported from the United States come from commercial turtle farms, however the exact number of turtles from these farms that were actually wild-caught individuals, used to supplement the breeding stock, is unknown, unreported, and unregulated (Colteaux & Johnson, 2017). The novel threat of overharvest for the sake of exportation, coupled with local harvest and habitat degradation, have serious conservation implications for the Southeastern United States, as it represents the second most biodiverse region for turtles in the world (Buhlmann et al., 2009). With the Asian turtle crisis decimating native abundances and diversity, the Southeastern United States is now arguably the most biodiverse region of turtles in the world.

The mobile drainage in Alabama is North America's biodiversity hotspot (Buhlmann et al., 2009), as the state boasts a total of 33 turtle species. However, the neighboring state of Mississippi likewise has substantial diversity, with 31 species within its range, some of which are endemic to certain river drainages and can only be found within the state. Even with this high diversity, very few statewide surveys have been completed on species other than *Graptemys* (Selman & Qualls, 2009; Lindeman, 1999). With the looming threat of species exploitation and exportation, possessing baseline data throughout the state is crucial. To allow for more informed management decisions, and for the better protection of our native turtle species, it is imperative that surveys throughout Mississippi are completed to document the species present, their distribution, relative abundance, habitat requirements, and community structure.

1.2 Riverine Species Description

1.2.1 Family Chelydridae

Chelydridae is a New World family of aquatic turtles commonly referred to as Snapping turtles. While *Chelydridae* is one of the oldest turtle families (Holman, 1995), only two genera remain (*Chelydra* and *Macrochelys*). Of these genera only five species of *Chelydridae* exist, three of which are found exclusively within North America, with two species found within the state of Mississippi (Turtle Taxonomy Working Group, 2017).

1.2.1.1 Genus *Chelydra* – 1 species

Worldwide there is a total of three species of *Chelydra*, however only one species can be found in North America. The North American Snapping turtle (*Chelydra serpentina*) (Photo 1), commonly referred to as the Eastern or Common Snapping turtle, dominates an extensive range from the East coast to the Midwest, and from Southern Canada to as far south as Florida’s peninsula and southern Texas (Turtle Taxonomy Working Group, 2017). This species is a fierce stocky turtle that gets considerably larger in the northern portion of its range where it does not compete with the Alligator Snapping turtle. *Chelydra serpentina* have a carapace with three sets of low keels and a highly reduced plastron. The head of the North American Snapping turtle is much smaller than that of the Alligator Snapping turtle, but they do share an extremely sharp, hooked beak and a powerful bite, with *C. serpentina* able to extend its neck much farther than its larger cousin.

This species can survive in almost any kind of freshwater habitat, from larger rivers to roadside cow ponds (Ernst & Lovich, 2009), and it can likewise consume almost anything. This omnivorous species has been documented eating freshwater sponges, numerous types of invertebrates from worms and mollusks to insects, crustaceans, and arachnids, to fish, frogs, toads, carrion, and algae (Ernst & Lovich, 2009). However, we've observed in regions where Alligator Snapping turtles are present, they seem to be less abundant within large water bodies, both rivers and oxbows, showing the possibility of competitive exclusion occurring between these two species.

1.2.1.2 Genus *Macrochelys* – 1 species

Alligator Snapping turtles are the largest freshwater turtle in North America and are restricted entirely to the Southern/Mideastern U.S. (Ernst & Lovich, 2009). The exact number of species is currently being debated, with some claiming there are two genetically distinct species, the Western Alligator Snapping turtle (*Macrochelys temminckii)* (Photo 2) and the Suwannee Alligator Snapping turtle (*Macrochelys suwanniensis)* (The Turtle Taxonomy Working Group, 2017). Others argue that *M. temminckii* should be split further into a third genetically distinct species, the Apalachicola Alligator Snapping turtle (*Macrochelys apalachicolae)* (Thomas et al., 2014).

The Western Alligator Snapping turtle is the only *Macrochelys* that can be found in Mississippi, and within the state, they are found in every drainage, in both riverine and oxbow habitats. Alligator Snapping turtles are an apex predator within their range, with only Alligators (*Alligator mississippiensis*) rivaling their size. They are known primarily as carnivores, with fish, salamanders, turtles, snakes, alligators, birds, and even mammals documented in stomach contents (Ernst & Lovich, 2009), but they are also known to regularly consume carrion, vegetation, fruits, and nuts (Ernst & Lovich, 2009).

While the historical range of *M. temminckii* extended through the Mississippi River north to Iowa, Illinois, and Indiana (Ernst & Lovich, 2009), due to a number of factors including habitat alterations and overharvest, the Western Alligator Snapping turtle's populations and range have been reduced dramatically (Riedle, et al., 2005; Shipman & Riedle, 2008, Jensen & Birkhead, 2003).

1.2.2 Family Emydidae

The emydid family is generally classified as semiaquatic pond and marsh turtles, with a few species designated as primarily terrestrial. The family is widespread, including modern species in the Americas, Europe, and Africa, and fossil records indicate an even greater historical range throughout much of Europe (Ernst & Lovich, 2009). Currently there are 11 genera and 32 species extant within North America (Stephens and Wiens, 2003), and of these, 7 genera and 17 species can be found within Mississippi, 12 of which are designated as riverine turtles.

1.2.2.1 Genus *Chrysemys* – 1 species

There is one species of *Chrysemys* within North America (Ernst, 1971); the Painted turtle (*Chrysemys picta*). One subspecies, the Southern Painted turtle (*Chrysemys* *picta dorsalis*) (Photo 3) is found within Mississippi. *Chrysemys picta dorsalis* can be distinguished from the other three subspecies of C. *picta,* by the single vertebral stripe on the dorsal portion of the carapace. This species can be found throughout the Southeast within river systems of Tennessee, Missouri, Arkansas, Louisiana, Alabama, and Mississippi (Powell, Conant, & Collins, 2016). Likewise, *C. p. dorsalis* has been reported in every river drainage of Mississippi, however it is much more prevalent in the central to northern portions of the state, with very low densities in the upper sections of the Pascagoula drainage, and no populations in the south.

Chrysemys picta dorsalis has been observed to bask year-round (Cagle, 1954), and can usually be found in slow-moving shallow-water habitats that possess soft bottoms, aquatic vegetation, and abundant basking sites (Ernst & Lovich, 2009). While the species is known to avoid fast currents, it can be found in both rivers and creeks (Ernst & Lovich, 2009).

1.2.2.2 Genus *Graptemys* – 9 species

Map turtle and Sawback are the common names for those species within the genus *Graptemys.* There are 14 recognized species within this genus (Powell et al., 2016), which can be characterized by a high level of river-drainage endemism (Lindeman[b], 1998). Of these 14 species, a total of nine can be found in Mississippi. Two species in particular are endemic to the Pascagoula River drainage which is entirely restricted to the state of Mississippi, and therefore these turtles can be found nowhere else in the world. These two species are the Pascagoula River Map Turtle (*Graptemys gibbonsi*) (Photo 4a) and the Yellow-blotched Sawback (*Graptemys flavimaculata*) (Photo 4b).

Likewise, the Black-knobbed Sawback (*Graptemys nigrinoda*), and the Alabama Map Turtle (*Graptemys pulchra*) are endemic to the Mobile River Drainage. While the Ringed Sawback (*Graptemys oculifera*) (Photo 4c), and the Pearl River Map Turtle (*Graptemys pearlensis)* (Photo 4d) are endemic to the Pearl River Drainage. However, these drainages are not restricted entirely to the state of Mississippi. As the Mobile River Drainage, stretches from Northwest Georgia and Northeast Mississippi into Alabama, where it then travels south. While the Pearl River drainage begins in Central Mississippi and travels Southwest to the border of Louisiana. Therefore, while endemic to one system, these turtles can be found in multiple states.

The three remaining *Graptemys* species, the Northern Map turtle (*Graptemys geographica*) (Photo 4e), the False Map turtle (*Graptemys pseudogeographica*) (Photo 4f & 4g), and the Ouachita Map turtle (*Graptemys ouachitensis*) (Photo 4h), can be found in numerous drainages throughout the Eastern United States, with both the Ouachita and Northern Map turtles reaching as far north as Canada (Powell, Conant, & Collins, 2016).

1.2.2.3 Genus *Pseudemys* – 1 species

There are ten species of Cooter, which make up the *Pseudemys* genus. They can be found throughout the eastern U.S., with one species reaching as far west as western Texas and New Mexico (Ernst & Lovich, 2009). However, there are only two species within the state of Mississippi, the Alabama Red-bellied Cooter (*Pseudemys alabamensis*) and the River Cooter (*Pseudemys concinna*) (Photo 5)). However, *P. alabamensis* is a habitat specialist, living in the brackish marshes along the coast, and therefore for the purpose of our study, this species has not been designated as a freshwater species and will not be discussed.

Pseudemys concinna on the other hand are a highly riverine, highly herbivorous species, that can reach sizes of around 260 mm carapace length (CL) for males, and 325 mm CL for females (Aresco & Dobie, 2000), with larger individuals seemingly more common where their range overlaps with the American Alligator (*Alligator mississippiensis*). As the common name suggests, *P. concinna* is an inhabitant of larger river and stream systems, preferring those with a moderate to fast current (Ernst & Lovich, 2009). However, the species can still be found in other large bodies of water such as ponds and oxbow lakes. *Pseudemys concinna* have an extremely large range, spanning from the East Coast west to Texas, Oklahoma, and Kansas, and as far north as Maryland and south to the panhandle of Florida (Ernst, 1997). Their broad range translates to Mississippi as well, as they can be found in high abundances across the state.

1.2.2.4 Genus *Trachemys* – 1 species

There are two species of Slider in the United States, the Big Bend Slider (*Trachemys gaigeae*), which has a range restricted to the Rio Grande Valley in the Big Bend region of Texas and Southcentral New Mexico, and the Pond Slider (*Trachemys scripta*) (Photo 6) whose original range was extensive and the species could be found throughout the Southeast and Southcentral U.S. (Powell, Conant, & Collins, 2016). Presently, however, due to the exportation for pet trade purposes, *T. scripta* can be found, sometimes in great numbers, on most every continent (Bringsoe, 2006; Pendelbury, 2007; Warwick, 1991). *Trachemys scripta* are opportunistic omnivores, with a wide-ranging diet of various plants, animals, and carrion (Ernst & Lovich, 2009). This diverse diet could be a key factor that allows the species to successfully survive in a multitude of habitats on numerous continents.

Trachemys scripta is split into two subspecies, the Yellow-bellied Slider (*T. s. scripta*), and the Red-eared Slider (*T. s. elegans*). The majority of the slow-moving systems or oxbow lakes in the state of Mississippi are dominated by the *T. s. elegans* subspecies, however in Southeast Mississippi where range maps show *T. s. elegans* exclusively, not only do many of the individuals lack the diagnostic post-orbital red stripe, but some possess the immaculate yellow plastron or the yellow blotch behind the eye, both of which are traits of *T. s. scripta.* This could be attributed to individual variation or a possible intergradation zone.

1.2.3 Family Kinosternidae

The family *Kinosternidae* consists of 25 species ranging throughout the New World (Ernst & Lovich, 2009). It is split into two genera; *Kinosternon*, the mud turtles, and *Sternotherus,* the musk turtles. Kinosternids possess musk glands that are present on either a single (*Sternotherus)* or a double-hinged plastron (*Kinosternon),* which excrete a malodorous musk (Iverson, Le, & Ingram, 2013). Of the 25-known species, four can be found within the state of Mississippi (Turtle Taxonomy Working Group, 2017).

1.2.3.1 Genus *Sternotherus* – 3 species

Of the 27 species that make up the Kinosternid family, presently only 6 belong to the genus *Sternotherus,* the musk turtles. Of these six, three can be found within the state of Mississippi, the Razor-backed Musk turtle (*Sternotherus carinatus)* (Photo 7a), the Common Musk turtle (*Sternotherus odoratus)* (Photo 7b), and the Stripe-necked Musk turtle (*Sternotherus peltifer)* (Photo 7c) (Turtle Taxonomy Working Group, 2017).

Sternotherus carinatus can be differentiated from the similar *S. peltifer* by its prominent vertebral keel and the dark speckling around the head and neck. It is found in

Texas, Oklahoma, Arkansas, Louisiana, Alabama, and Mississippi (Turtle Taxonomy Working Group, 2017). *Sternotherus carinatus* prefers rivers, streams, oxbows, and swampy habitats that possess muddy bottoms, aquatic vegetation, and basking structure (Ernst & Lovich, 2009). Within Mississippi, *S. carinatus* can be found within the Pascagoula and Pearl River drainages, and likewise into portions of the Yazoo River (Turtle Taxonomy Working Group, 2017).

Sternotherus odoratus also known as the Common musk turtle, or Stinkpot, due to its characteristic musky scent, has a much wider distribution compared to any other species of *Sternotherus,* ranging from mid-Texas to the Great Lakes, Canada, and Maine. A large gap exists between the east coast and western populations due to the Appalachian Mountains (Turtle Taxonomy Working Group, 2017). However, it can be found throughout the entire state of Mississippi, and as a habitat generalist it can be found in any sort of aquatic habitat (Ernst & Lovich, 2009). *Sternotherus odoratus* can be distinguished by the two large supra- and infra-orbital stripes that begin at the nares and continue onto the neck region.

The final *Sternotherus* that can be found in Mississippi is *Sternotherus peltifer*. This turtle was designated as a subspecies of the loggerhead musk turtle for some time, however recently it has been proposed that *S. minor* is actually three distinct species, *S. minor*, *S. peltifer,* and *S. intermedius* (Scott et al., 2018). *Sternotherus peltifer* has a keel, similar to that of *S. carinatus*, however the overall slope of its shell is much more gradual, and it possesses a striped pattern on both the face and neck (Powell, Conant, & Collins, 2016). The species can be found in Alabama, Georgia, Mississippi, Tennessee, Kentucky, and Virginia (Turtle Taxonomy Working Group, 2017).

1.2.4 Family Trionychidae

The family *Trionychidae*, commonly known as softshells, are simultaneously genetically and geographically diverse. There are 10 genera within the family, and a total of 23 species (Turtle Taxonomy Working Group, 2017). Trionychids can be found across the globe, in locations including Asia, the Middle East, Africa, the Pacific islands, and North America (Turtle Taxonomy Working Group, 2017).

1.2.4.1 Genus *Apalone* – 3 species

Of the ten genera, only *Apalone* can be found within North America. There are three species within the genus *Apalone,* two are found within the state of Mississippi, the Spiny Softshell (*Apalone spinifera)* (Photo 8a) and the Smooth Softshell (*Apalone mutica)* (Photo 8b).

Apalone spinifera is a widespread turtle species spanning much of the South and Mid-Eastern United States, into both Southern Canada and Northern Mexico, with introduced populations popping up across the Midwest (Turtle Taxonomy Working Group, 2017). There are six designated subspecies of the Spiny softshell, with the Northern spiny softshell (*A. s. spinifera)* covering the majority of the species range, including the northern portions of Mississippi. The Gulf Coast Spiny Softshell (*A. s. aspera*) inhabits the southeastern portion of the state, while the Mississippi River is the eastern extent of the Pallid Spiny Softshells (*A. s. pallida)* range. There is a possibility of overlap between three of the *A. spinifera* subspecies within the southwestern portions of Mississippi (Turtle Taxonomy Working Group, 2017).

The Spiny Softshell shows extreme sexual dimorphism, with adult females $(\text{Straight Line Caprapace Length } (SCL)_{max}$, 54.0 cm) reaching sizes that are on average 1.6 times larger than adult males (SCL_{max} , 21.6 m) (Photo 8c) (Graham & Cobb, 1998). Likewise, adult female's carapace markings become mottled or blotched, while males retain the clean circular pattern seen in juveniles. *Apalone spinifera* can be distinguished from the overall similar *Apalone mutica* by the presence of cutaneous "spines" along the anterior edge of the carapace (Ernst & Lovich, 2009). These spines can be somewhat large and conical, the norm for larger females, or extremely small, with the texture of sandpaper, more common in smaller individuals or males.

Apalone mutica, like *A. spinifera,* is a widely distributed species, found throughout the central United States. This species ranges through the entire Mississippi River drainage, as well as separate drainages in Texas, Mississippi, and Alabama (Turtle Taxonomy Working Group, 2017). There are two subspecies of Smooth softshell, the Midland smooth softshell (*A. m. mutica)* and the Gulf Coast Smooth Softhshell (*A. m. calvata). Apalone mutica calvata* has a much more limited range compared to *A. m. mutica*, inhabiting only the Pearl and Pascagoula drainages in Mississippi, and the Mobile Drainage of Alabama (Turtle Taxonomy Working Group, 2017). *Apalone mutica* is smaller than *Apalone spinifera* with an average SCL_{max} of 35.6 cm in females, and 26.6 cm in males (Moler, 2006), and prefers larger rivers and streams than does the spiny softshell (Dreslik & Philips, 2005).

1.3 Figures

Figure 1.1 North American Snapping turtle (*Chelydra serpentina)*

Figure 1.2 *Macrochelys temminckii* (Western Alligator Snapping turtle)

Figure 1.3 *Chrysemys picta dorsalis* (Juvenile Southern Painted turtle)

Figure 1.4 *Graptemys* species

a. young male Pascagoula Map Turtle (Graptemys gibbonsi), b. young male Yellow-blotched Sawback (Graptemys flavimaculata), c. juvenile Ringed Sawback (Graptemys oculifera), d. adult female Pearl River Map turtle (Graptemys pearlensis), e. male Northern Map Turtle (Graptemys geographica), f. Mississippi Map Turtle (Graptemys pseudogeographica kohnii), g. Northern False Map turtle (Graptemys pseudogeographica pseudogeographica), & h. Ouachita Map turtle (Graptemys ouachitensis).

Figure 1.5 Pseudemys concinna (River Cooter).

Figure 1.6 Trachemys scripta elegans (Pond Slider).

Figure 1.7 *Sternotherus* species

7a. Razor-backed Musk turtle (Sternotherus carinatus), 7b. A Stinkpot or Common Musk turtle (Sternotherus odoratus), & 7c. A Stripe-necked Musk turtle (Sternotherus peltifer).

Figure 1.8 *Apalone* species

8a. male Spiny Softshell turtle (Apalone spinifera), 8b. male Smooth Softshell turtle (Apalone mutica), & 8c. sexual dimorphism in A. spinifera with an adult male on the left, and an adult female on the right.

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CHAPTER II – THE BREAKDOWN OF SAMPLED SYSTEMS AND RIVERINE TURTLE DISTRIBUTIONS AND ABUNDANCES, IN SOUTHERN MISSISSIPPI

2.1 Introduction

Of the 356 known extant species of turtles, 149 (42.8%) are currently considered threatened and 84 (24.1%) are considered endangered or critically endangered (Rhodin et al., 2017). This makes turtles the most threatened vertebrate group in existence today, surpassing even amphibians and primates (Lovich et al., 2018). The Southeastern United States is one of two global hotspots for turtle biodiversity (Buhlman et al., 2009), including the state of Mississippi. Although Mississippi has over 30 species, there has been very few turtle studies or surveys done within the state. This lack of research is even more startling due to a number of factors, including habitat degradation, fragmentation and destruction, and harvest for food or the pet trade, which are causing the population decline, or even extirpation, of numerous turtle species across the world (Fund, T.C., 2002). Due to the aging threat of legal harvest, and the novel threat of illegal exportation, turtles are a group that needs attention. While there has been no North American turtle crisis, the over exploitation of native species has historically occurred and, in some places, continues. Therefore, it is imperative we have accurate and robust data characterizing these communities and populations. This is especially true for Mississippi which has never before had a comprehensive survey done within the state.

The overarching goal of this project is to perform this survey and document the diverse species richness present here, allowing future researchers to track changes over time, and officials to make more informed management decisions. Understanding the turtle diversity across the state is a critical step in better understanding how to protect our native turtles in this local biodiversity hotspot as we move into the future.

2.2 Methods

Our surveys focused on the two main southern drainages of Mississippi, the eastern Pascagoula River drainage, and the western Pearl River drainage, with a brief three site survey along the Big Black River, and a single survey on the Jourdan River (Fig. 2.1). During the 2017 season twelve sites were surveyed within the Pascagoula River drainage, these included four sites along the Chickasawhay, four sites along the Leaf River, two sites along the Bouie, and two sites along the Pascagoula River Proper (Table 2.1 & Fig. 2.2). These included six sites designated as riverine (lotic), and six sites designated as lentic, which consisted of oxbow lakes. During the 2018 season fourteen sites were surveyed within the Pearl River drainage these included two sites in the Upper Pearl River, five sites around the Ross Barnett Reservoir area, three sites in the Middle Pearl River, three sites in the Lower Pearl River, and a single site on the Bogue Chitto (Table 2.1 & Fig. 2.3), a large Pearl River tributary. Four of these sites were designated as lentic, which consisted of oxbow lakes, sloughs, backwaters, and the reservoir, while the remaining ten were designated as riverine. In addition, three sites along the Big Black River (Table 2.1 & Fig. 2.4), and a single site along the Jourdan River (Table 2.1 & Fig. 2.3) were surveyed during this season. Each water body or stretch of river where approximately 23 nets were set, baited, and checked, over a three to four-day period, constitutes a “site” in all subsequent analyses and results.

These baited hoop nets (90 cm diameter, 3-metal ring, and 120 cm diameter, 7-fiberglass ring) were partially submerged near suitable microhabitat (log jams, root masses, etc.) with the trap tied to structure and/or a PVC pipe secured into the substrate. Traps were baited with frozen or fresh fish, bait type was recorded, and traps were checked within 24 hours of setting, for a total of two to three check days. All captured turtles were identified to species, sex was determined, and all were uniquely marked. *Apalone* were marked initially using a unique combination of biopsy punches (Miltex 4 mm diameter), on the posterior carapace, but due to numbering constraints we changed this marking system to unique tattoo IDs using a battery-operated tattooing gun (Inkinator cordless) (Weber et al, 2011). *M. temminckii* were marked using a unique combination of notches on marginal scutes 8 – 12. All remaining turtle species were marked using the Ernst notching method (Ernst, Hershey, & Barbour, 1974). Tissue (webbing from hind foot, tail tip of less than 5 mm, or carapace biopsy punches for *Apalone*) were acquired to create a genetic bank of all turtle species for possible future genetics studies. Tissue was not taken from *M. temminckii,* instead blood was collected from the dorsal coccygeal vein for both a DNA sample and basic health assessments, and claw tips were collected to assess chronic mercury concentrations. Morphometric measurements (cm) were recorded, and included straight-line carapace length, width, height, plastron length, and mass (g). Likewise, anecdotal data such as injury, location of injury, and presence of leeches were recorded. Turtles were then released at the point of capture.

On days that traps were checked opportunistic sight surveys were conducted from the front of the boat with binoculars. These surveys were included to target non-piscivorous species that were rarely captured in baited traps (e.g., *Graptemys gibbonsi* and *G. flavimaculata*), or to take note of species that are present at a site, but were not captured. On river sites, surveys were started at the boat launching point and completed when we arrived at the starting trap location, this reduced the likelihood of counting an individual twice and allowed us to determine basking abundance per river kilometer (BA $= \frac{\#\ of\ individuals\ of\ a\ single\ species\ observed}{Total\ River\ km\ traveled}$). At lake sites where travel is generally non-linear site surveys were done opportunistically and used only to determine species presence/absence. Air temperature, general weather conditions, and turtle abundance were recorded, and, where possible, turtles were identified to species. During these surveys' other external factors such as boat traffic (number of boats along stretch of river or lake), number of limb lines and trot lines, and number of alligators were also recorded. The number of river kilometers that were covered during basking surveys were determined by measuring the river channel from the point of launch to our starting trap using Google Earth.

2.2.1 River Drainage Analysis

When comparing the number of individuals captured, Simpson's Diversity ($D = 1 - \frac{\Sigma\, n(n-1}{N(N-1)}$), Simpson's Equitability index ($E_D = 1 - \frac{\Sigma\, n(n-1}{N(N-1)} \times \frac{1}{S}$) , and richness across our three main systems (Pascagoula River Drainage, Pearl River Drainage, and Big Black River) a One Factor ANOVA with system as a fixed factor was used if the parametric test assumptions of normality (Sharpiro-Wilk goodness of fit test) and equal variances (Bartlett's test) were met. If the ANOVA yielded a significant difference, a Tukey's HSD Post Hoc test was performed. If these assumptions were not met a Kruskal-Wallis Rank sum test was performed, and if significant differences arose, a Wilcoxon each pair test was performed. To make these comparisons across only two river systems, or between lake and river sites, a pooled variance two sample, two tailed t-test was performed if all test assumptions were met. If either the assumption of equal variances or that of normality were not met, either an Unpooled two sample two tailed t-test, or a Wilcoxon rank sum test, was performed, respectively. A Contingency table Analysis was used to compare the frequencies of leeches on individuals captured from riverine sites, to those captured lake sites. We likewise compared the relative abundance of each species, and total number of turtles, to the amount of fishing pressure (number of lines present) at each site, using an analysis of covariance (ANCOVA), with species as a factor, the number of lines as a covariate, and abundance as the dependent variable. All statistical analyses were conducted using JMP software.

2.2.2 Species Analysis

Relative abundance of all captured species ($RA = \frac{\#\ individuals\ from\ one\ species}{\#\ individuals\ from\ all\ species\ present}$) was calculated at each site, as well as each overall drainage. Likewise Catch per Unit Effort (CPUE, CPUE = $\frac{\#\ turtles\ captured}{\#\ trap\ nights}$), which refers to the likelihood of capturing a single turtle in a single trap night, was calculated for each captured species at each trap site, per river system, and for the entirety of the survey. Chi-square (X^2) contingency table tests were used to determine if the observed sex ratio for each species within each system, and overall, differed significantly from 1:1. We used a Contingency table Analysis to compare the frequencies of leeches, and injuries, across species. To compare catch rates of a single species, the total turtles captured, turtles captured per day, and CPUE, across the three main systems a One Factor ANOVA with system as a fixed factor was used if all parametric test assumptions were met. When a significant different arose, a Tukey’s HSD Post Hoc test was performed. If all assumptions were not met a Kruskal-Wallis Rank sum test was performed, and if significant differences arose, Wilcoxon each pair test was performed. If only two drainages could be compared a Pooled two sample two tailed t-test was performed if all test assumptions were met. If test assumptions were violated either an Unpooled two sample two tailed t-test, or a Wilcoxon rank sum test, was performed. To compare capture rates of a single species, total catch rates, size differences, and CPUE across lake sites and river sites a Pooled two sample two tailed t-test was performed if all test parameters were met. If certain parameters were not met either an Unpooled two sample two tailed t-test, or a Wilcoxon rank sum test, was performed.

2.3 Results/ Discussion

In total, we captured 1,230 individuals representing 16 species (Table 2.2, 2.3, & 2.4). Thirty sites were surveyed along the Pascagoula River Drainage (12) (Fig. 2.2), Pearl River Drainage (14) (Fig. 2.3), Big Black River (2.4) (Fig. 4), and a single site on the Jourdan River (Fig. 2.3), for a total of 1,898 trap nights. All sites combined had a CPUE of 0.644 turtles per trap night. On average, we caught significantly more turtles per day ($F_{2,90} = 6.738$, $p = 0.0019$) at our 3 Big Black River sites ($\bar{x} = 17.8$ SD = 10.5, N = 9) and 11 Pascagoula Sites ($\bar{x} = 13.7$, SD = 11.5, N = 44) compared to our 14 Pearl Sites ($\bar{x} = 9.25$, SD = 7.4, N = 40). Overall, river sites ($\bar{x} = 6.84$, SD = 1.26, N = 19) had significantly greater species richness ($t = 4.70$, df = 27, $p < 0.0001$) than lake sites ($\bar{x} = 4.70$, SD = 1.16, N = 10). The river sites within the Pascagoula River ($\bar{x} = 7.8$, SD = 0.408, N = 6) had significantly higher species richness ($\chi^2 = 8.31$, df = 2, $p = 0.0157$) than both the Big Black River ($\bar{x} = 6.3$, SD = 1.15, N = 3), and the Pearl River ($\bar{x} = 6.4$, SD = 1.35, N = 10).

2.3.1 Drainage Description

2.3.1.1 Pascagoula River Drainage

With a drainage area of about 25,123 km^2 (U.S. Army Corps of Engineers, 1968), the Pascagoula River systems is the largest unimpounded drainage in the contiguous United States (Dynesius and Nilsson, 1994). Previous studies have found this system to be a stronghold for, riverine fish (Heise, Slack, and Ross, 2004) as there are few human alterations that affect water temperature or flow. We believe it’s possible similar claims could likewise be made for freshwater turtles.

A total of 646 individual turtles from 11 species were captured from 12 sites along the Pascagoula River from May to September 2017, with an average of 6 species per site (Table 2.2 & 2.5). When we compare river sites to lake sites, we find the average species richness at river sites ($\bar{x} = 7.83, SD = 0.477, N = 6$) is significantly greater (χ^2 = 2.92, p = 0.0035) than that of lake sites ($\bar{x} = 4.17, SD = 1.169, N = 6$). The greatest number of species observed at a site was 8, which occurred at all river sites except for Pascagoula Site 9. The lowest number of species observed per site was 3, which occurred at both Pascagoula Sites 5 and 11. The most individuals caught at a single site was at Pascagoula Site 4, this site was located on private property and a total of 160 individual turtles were captured. However, 85% of these turtles were *T. scripta*, and this skew towards one species is represented in the evenness score of the site (E_D = 0.23), which is likewise the lowest among all Pascagoula Sites.

Similar to the overall species richness measures, a definite pattern is present in Simpsons Diversity Index (D), and Simpsons Equitability Index (E_D) (Table 2.6).

Simpson's Diversity was significantly greater ($t = 10.13$, DF = 10, $p < 0.0001$) at river sites ($\bar{x} = 4.89, SD = 0.593, N = 6$) compared to lake sites ($\bar{x} = 1.55, SD = 0.239, N = 6$). Similarly, the evenness was significantly greater ($t = 2.619$, DF = 10, $p = 0.0256$), at river sites ($\bar{x} = 0.5867, SD = 0.076, N = 6$), compared to lake sites ($\bar{x} = 0.415, SD = 0.142, N = 6$). The evenness of river sites ranged from 0.68 at Pascagoula Site 7, with a turtle community composed of mostly *T. scripta* (RA = 0.3038) and *A. spinifera* (RA = 0.2785), to 0.47 at Pascagoula Site 8, which was heavily dominated by *A. spinifera* (RA = 0.3793) and *M. temminckii* (RA = 0.3103). Lake sites ranged from 0.62 E_D at Pascagoula Site 10 site, which produced three species (*A. spinifera, M. temminckii*, and *S. carinatus*) and 16 individuals, to the previously mentioned Pascagoula Site 4 ($E_D = 0.23$), which produced six species (*C. serpentina, M. temminckii, P. concinna, S. carinatus, S. odoratus,* and *T. scripta*) and 160 individuals, but again was dominated by *T. scripta*.

Certain species, such as *A. mutica*, *C. serpentina*, *S. odoratus*, and *S. peltifer*, were relatively scarce during our surveys (Table 2.5), as they were rarely captured or recorded basking. It is likely our surveys were not in the proper habitat for some species (*C. serpentina* and *S. peltifer*), and surveys of smaller lakes or creeks would yield higher capture rates. Many sites most likely possessed *S. odoratus*; anecdotally, we observed individuals basking or crossing roads. However, due to their extremely small size it is likely they were unable to enter our traps, or were able to simply slip out, therefore avoiding detection. While other species like *A. mutica* seem to generally have low capture rates (Riedle, 2015; Dreslik, et al., 2005), they will readily go to traps (Anderson, et al., 2002). While catchability likely differed among species, these differences were presumably present in each sampled location, so our relative abundances, and can still be meaningfully compared across sites or drainages.

On the other hand, numerous species were abundant throughout the system. Both species of endemic *Graptemys*, the *G. gibbonsi* and the *g. flavimaculata*, were present at every river site, and the Pascagoula Sites 11 and 12, which were classified as lentic. *Pseudemys concinna* was captured at every river site and Pascagoula sites 4 and 12, with the most individuals (15) caught at Pascagoula Site 2. Of the musk turtles (*Sternotherus)*, *S. carinatus* was by far the most abundant with a total of 33 individuals captured from seven sites, compared to only two *S. peltifer* both captured at the Pascagoula Site 2, and 5 *S. odoratus* captured from 3 lentic sites.

By far the most abundant species were *A. spinifera* (RA = 0.149), *M. temminckii* (RA = 0.164), and *T. scripta* (RA = 0.488). However, these abundances do shift when we look only at river or lake sites (Table 2.7), with *A. spinifera* abundances plummeting at lake sites ($RA_{LAKE} = 0.02$, $RA_{RIVER} = 0.25$), and *T. scripta* showing the opposite pattern ($RA_{LAKE} = 0.79$, $RA_{RIVER} = 0.11$). While *M. temminckii* abundances remained relatively constant from river to lake sites ($RA_{LAKE} = 0.18$, $RA_{RIVER} = 0.12$). It should be noted that *A. spinifera* and *M. temminckii* are both highly piscivorous, which may inflate their capture rates compared to more omnivorous or herbivorous species.

2.3.1.2 Pearl River Drainage

From its headwaters in east central Mississippi, the Pearl River runs west to Jackson and then south to become the border between MS and Louisiana, with a drainage area of approximately 22,688 km^2 (Rogillio, et. al, 2007). Unlike the Pascagoula River, which has had very little human impact, the Pearl River has experienced substantial disturbances since the 1950s, including the Ross Barnett Reservoir construction, addition of a navigation channel, and channel modifications of the river's main stem (Piller, et. al., 2004). Numerous studies have shown the effects of modifications such as these on fishes to vary depending on the species, and the habitats they occupy; midwater or surface habitat fish tend to show little decline (Williams et al. 1989; Warren and Burr 1994; Etnier 1997), compared to benthic fishes which seem to be most affected (Warren and Burr 1994; Warren et al. 2000). But few studies have looked at the possible effects of such river alterations on freshwater turtles.

A total of 388 individuals from 10 species were captured from 14 sites along the Pearl River from May to September 2018, with an average of 6 species per site (Table 2.3). Unlike the Pascagoula River, there was no significant difference (χ^2 = 2.75, p = 0.0973) between the species richness of river ($\bar{x} = 6.4, SD = 1.35, N = 10$) or lake sites ($\bar{x} = 5.5, SD = 0.577, N = 4$). The greatest number of species observed per site was 10 (Table 2.8), which occurred at Pearl Site 1 river site. This location was designated as a river site; however, its waters were relatively slow flowing and it was an extremely small stretch located in the headwaters of the Pearl River, with an average stream width of only 16.1 meters. The fact this site had characteristics of both a lentic and lotic ecosystem, could be the reason numerous species that are known to prefer lake habitats, such as the *C. serpentina* or the *C. p. dorsalis*, were present. Likewise, there were numerous swamp-like habitats directly upland of these sites, it is likely that during high water flash flooding events, individuals could be swept into the small streams. It is also highly likely that due to the site's small size, we were able to observe more of the turtle community than would be possible at a larger site. Therefore, it is probable our richness counts at other, larger sites inadvertently exclude species. The lowest number of species observed per site was 5, which occurred at Pearl Sites 5, 6, and 13.

The most individuals caught at a single site was at Pearl Site 11, with 71 individuals. But, similar to Pascagoula Site 4, a large number (76%) of these turtles were *T. scripta*, this skew towards one species is represented in the evenness score of the site (E_D = 0.36) which is likewise the lowest among all Pearl Sites (Table 2.9).

Similar to the overall species richness measures, and unlike the Pascagoula River, there is no significant difference (t = 1.638, DF = 12, p = 0.1274) in the Simpsons Diversity Index(D) between lakes ($\bar{x} = 2.27, SD = 0.797, N = 4$) and rivers ($\bar{x} = 3.50, SD = 1.4, N = 10$), and no significant difference (t = -0.3101, DF = 12, p = 0.7618) in Simpsons Equitability Index (E_D) between lakes ($\bar{x} = 0.679, SD = 0.22, N = 4$) and rivers ($\bar{x} = 0.643, SD = 0.19, N = 10$). The evenness of river sites ranged from 0.46 at Pearl Site 1 site in which we caught 30 individuals, mostly *T. scripta* (RA = 0.400) and Pearl River Map turtles (RA = 0.300), to 0.95 at Pearl Site 10, which only caught 20 individuals, the most abundant being *M. temminckii* (RA = 0.300). While the equitability of lake sites ranged from 0.47 at Pearl Site 6, which produced three species (*A. spinifera, C. serpentina*, and *T. scripta*) and 47 individuals, the great majority of which were *T. scripta* (RA = 0.83), to 0.99 at Pearl Site 5. This high level of evenness was not due to the capture of numerous individuals of many species, instead it was because we captured only nine individuals of two species (*M. temminckii* and *T. scripta*). While other species (*A. spinifera, G. oculifera,* and *G. Pearl Riverensis*) were observed to be present there, the catch rates were exceedingly low. This is similar to the Pearl Site 4, in which we captured only 16 individuals of 4 species (*A. spinifera, M. temminckii, P. concinna,* and *S. carinatus*). However, these sites varied considerably in surrounding land use, with Pearl Site 4 sprawling into swamps and backwaters with few houses, while Pearl Site 5 was surrounded by large developments, and established improved channels, which could have an effect on the turtle populations.

Similar to the Pascagoula River, certain species such as *A. mutica, C. serpentina, S. odoratus,* and *C. p. dorsalis,* were rarely captured or recorded basking during our surveys (Table 2.8). It is likely our surveys were not in the proper habitat for some species (*C. serpentina* and *C. p. dorsalis*), because when we did trap smaller sloughs or sites adjacent to small lentic habitats these species were captured or observed more frequently. Therefore, surveys of smaller lakes or creeks would most likely yield higher capture rates. Likewise, many sites most likely possessed *S. odoratus*. Anecdotally, we again observed individuals basking or crossing roads, however due to their extremely small size it is likely they were unable to enter our traps, or were able to simply slip out, therefore avoiding detection.

One of the most surprising observations about the Pearl River, was the seemingly complete lack of *A. mutica*. No individuals were captured in 625 trap nights at river sites. And only one individual was overserved during basking surveys, at Pearl Site 10. The fact that we captured individuals of this species at all but one Pascagoula Site, and all Big Black River sites, suggests that if the species is present in any sort of number at least one individual will be captured. There may be such extremely low abundances within the

Pearl River, that we were simply not able to capture any individuals. Historically, there are records of individuals throughout the Pearl River, all South of the Ross Barnett Reservoir, so the species was present, and is highly likely to remain present within the system. However, a majority of these records were from prior to the 1980s, with only 9 taking place after 2000. Due to the anecdotal and qualitative nature of these historical records it is impossible to determine whether this river has suffered a decline in *A. mutica*, or if the species has always been sparse within the Pearl River.

Similar to the Pascagoula River, the most abundant species were *A. spinifera* (RA = 0.11), *M. temminckii* (RA = 0.24), and *T. scripta* (RA = 0.39) (Table 2.10). Unlike the Pascagoula River, the relative abundance of *S. carinatus* was greater in the Pearl River (RA = 0.11). While we did catch 10 fewer individuals in the Pascagoula River, the main reason for this jump in relative abundance is the decline in capture rates of almost all other species, with the Pascagoula Sites ($\bar{x} = 53.83, SD = 0.37.86, N = 12$) on average catching significantly more turtles ($F_{2,26} = 4.46$, p = 0.0216) than Pearl Sites ($\bar{x} = 27.71, SD = 16.31, N = 14$). And while this is true in trap captures, when looking at the basking presence of the microcephalic *Graptemys* species, on average there were significantly more (*t* = 3.288, DF = 13, p = 0.0059) *G. oculifera* ($\bar{x}$ = 6.26 individuals/ 1 river km, SD = 7.137, N = 10) observed basking than *G. flavimaculata* ($\bar{x}$ = 0.969 individuals/ 1 river km, SD = 1.04, N = 6). However, this could be due to the fact we were able to trap numerous lower stretches on the Pearl River. Lower stretches along the Pascagoula River and Pearl Rivers have been shown to have larger abundances of microcephalic *Graptemys*, compared to upper stretches (Selman & Qualls, 2009). We were unable to trap similar lower stretches on the Pascagoula River due to constant rain and flooding events during the spring and summer of 2017. Likewise, an increase in boat traffic on the Pearl River, may have resulted in individuals that were less likely to bail off their basking platforms due to habituation, and thus we were able to count more.

It is hard to determine the reason for the significantly fewer turtles detected on the Pearl River. Not only are there numerous man-made or human impacted structures, but the drainage is likewise relatively linear. This is in opposition to the much more dendritic Pascagoula River, which could provide refugia for turtles during high water, or other events. Likewise, we saw increased river traffic, and fishing pressure on the Pearl River, but the analysis of covariance showed these do not seem to impact the number of turtles present ($F_{1,12} = 0.7432$, $p = 0.4055$). On the other hand, this is the first study to survey the systems of Southern Mississippi’s entire turtle community, it is likewise possible the abundances of turtles within the Pearl River has always been lower than that of the Pascagoula River.

2.3.1.3 Big Black River

The Big Black River runs approximately 434 km from the North Central Hills of central Mississippi southwest where it empties into the Mississippi River (Hartfield and Rummel, 1985). Unlike the Pearl River, the Big Black River remains an ecologically functional floodplain river system (Abell et al., 2000), and there have been relatively few human influences along its reach (Mareska and Jackson, 2002); however, there have been some human alterations. There are no dams within the main stem of the system, but some of the smaller tributary streams do have impoundments. Likewise, there have been minimal channel modifications for navigation and flood control which date back to the

1950s (U.S. Army Corps of Engineers, 1964). The Big Black River runs through a heavily rural area, with very few human population centers along its length (< 25 people/ km^2), and is mostly surrounded by forest (~54%), agriculture (~35%), and farmland (~11%) (Insaurralde, 1992). The relatively natural, unaltered state of the stream and watershed could be a reason for the highly abundant turtle populations present there.

A total of 165 individuals from 8 species were captured from 3 sites along the Big Black River (Table 2.4 & Fig. 2.4) from June to September 2018, with an average of 6.3 species per site (Table 2.11), and an average catch per unit effort (CPUE) of 0.8 turtles per trap night. The greatest number of species observed per site was 7, which occurred at Big Black Sites 2 and 3. The lowest number of species observed per site was 5, which occurred at Big Black Site 1. The most individuals caught was at Big Black Site 3, in which 78 individuals were captured. But, unlike the sites on the Pearl River and the Pascagoula River where a larger percentage of individuals were of a single species, Big Black Site 3 had a relatively high evenness score ($E_D = 0.53$) (Table 2.12). This reflects the fact that the species which had the highest relative abundance, *M. temminckii*, consisted of less than half of our overall captures (42.8%). Big Black site 3 still had the lowest evenness score when compared to other Big Black River sites, however, it was only slightly lower than Big Black Site 2 ($E_D = 0.56$), in which *M. temminckii* again were caught more than any other species (40.0%). Big Black Site 1 had the greatest evenness score ($E_D = 0.80$), due to the relatively similar catch rates among species, as we caught 13 individuals of both *M. temminckii* and *T. scripta* (31%), 7 individuals of both *A. spinifera* and *A. mutica* (17%), and 2 *G. pseudogeographica* (4%).

Similar to the situation in both the Pascagoula River and Pearl River, certain species, such as *C. serpentina*, *S. odoratus*, or *S. carinatus* were not captured or recorded basking during our surveys. Again, it is likely *C. serpentina* and *M. temminckii* compete for much of the same resources, and due to the high relative abundances of *M. temminckii*, *C. serpentina* have moved to occupy space in smaller rivers, lakes, or sloughs, in areas adjacent to the main stem. Unlike the Pascagoula River and the Pearl River, no *S. odoratus* were observed or captured at any sites. They have been recorded in two of the four counties our three trap sites were located in, however none of these records is from the Big Black River system. So, it is unknown if this species is present within the drainage at our trapping locations.

Another species that was relatively abundant in other large river systems that we did not capture or observe in the Big Black was *S. carinatus*. There are no historical records for *S. carinatus* within our sites, and while we captured individuals in Madison county, in which both Big Black Sites 2 and 3 are located, these records were from our Pearl Sites 3 and 4. The single Big Black River *S. carinatus* historical location (NMNH, 2016) is approximately 74 river km downstream of Big Black Site 3, and whether these records even correspond with a location on the Big Black River is questionable. The average catch per unit effort of *S. carinatus* on both the Pascagoula River ($\bar{x}_{\mathrm{CPUE}} = 0.062$) and Pearl River ($\bar{x}_{\mathrm{CPUE}} = 0.061$) was somewhat low when compared to other species. But, even if their abundance was drastically lower, to not capture or observe any individuals in 206 trap nights points to the possibility that for some reason *S. carinatus* may not inhabit the Big Black River, or at least not the portions that were surveyed.

A turtle that was surprisingly abundant on the Big Black River was *A. mutica*. Nineteen individuals were captured in 206 trap nights ($\bar{x}_{\text{CPUE}}$ = 0.092), which is 10 more individuals than the Pascagoula River ($\bar{x}_{\text{CPUE}}$ = 0.031) with far fewer trap nights recorded on the Big Black River. Opposite to the Pascagoula River, where we generally captured more males and juveniles, the majority of those captured on the Big Black River were female (16 individuals), with only one male and one juvenile captured. However, *A. mutica* were not the only species that was on average more relatively abundant, both *A. spinifera* and *M. temminckii* showed a greater relative abundance in the Big Black River (RA $\bar{x}_{\textit{A. spinifera}}$ = 0.226, RA $\bar{x}_{\textit{M. temminckii}}$ = 0.378) when compared to both the Pascagoula River (RA $\bar{x}_{\textit{A. spinifera}}$ = 0.165, RA $\bar{x}_{\textit{M. temminckii}}$ = 0.230) and the Pearl River (RA $\bar{x}_{\textit{A. spinifera}}$ = 0.110, RA $\bar{x}_{\textit{M. temminckii}}$ = 0.316).

Unlike the Pascagoula River and the Pearl Rivers, there are no Map turtle species that are endemic to the Big Black River system, however that does not mean it is devoid of *Graptemys*. We captured two species, the Ouachita Map turtle (*G. ouachitensis)* and 2 subspecies of the False Map turtle, the Northern False Map turtle (*G. pseudogeographica pseudogeographica*) and the Mississippi Map turtle (*G. p. kohnii)* as the Big Black River is located in a region of overlap and intergrades (Rhodin, et al., 2017). Basking counts for *Graptemys* were lumped into a single category, as *G. ouachitensis* and *G. pseudogeographica*, including the sub species, are extremely hard to differentiate through binoculars while on a moving boat. An average of 2.23 individuals were observed basking per river km, which was fewer than combined counts of *G. gibbonsi* and *G. flavimaculata* on the Pascagoula River ($\bar{x}$ = 2.97 individuals per river km) and *G. pearlensis* and *G. oculifera* on the Pearl River ($\bar{x}$ = 8.29 individuals per river km). The sites surveyed were relatively far upstream, and the number of basking *Graptemys* could increase as drainage area increases.

While the basking surveys may show a smaller number of individuals overall, the catch per unit effort of *G. pseudogeographica* on the Big Black River ($\bar{x}_{\mathrm{CPUE}} = 0.061$) is similar to that of *G. pearlensis* on the Pearl River ($\bar{x}_{\mathrm{CPUE}} = 0.061$), but less than *G. gibbonsi* on the Pascagoula River ($\bar{x}_{\mathrm{CPUE}} = 0.093$). Unlike *G. pseudogeographica*, *G. ouachitensis* was much less abundant, and we caught only a single individual at Big Black Site 3. The Big Black River seems to be on the western edge of *G. ouachitensis* range possibly causing small populations (Rhodin, et al., 2017). As surveys continue down river and towards the west, we will better observe if these wide ranging *Graptemys* species become more abundant as drainage area increases, and as we exit the periphery of their range.

Very little work has been done on the turtle species and communities within the Big Black River. The 3 sites we completed this year are a good baseline. However, as more work is completed in future trapping seasons, more realistic and reliable population distribution and abundance estimations can be made. Likewise, as trap nights increase, we will have more of a reliable idea of the presence of certain species, like the *S. carinatus*.

2.3.1.4 Jourdan River

The Jourdan River is one of the main tributaries of the St. Louis Bay. Together with the Wolf, St. Louis Bay's second main tributary, they drain an area of approximately 790 mi^2 (Suttkus, et al., 1998). We trapped a single location along the Jourdan River

(Table 2.1 & Fig. 2.3) (Site 15), which was approximately 23.6 river km from the Bay of St. Louis, and was extremely tidally influenced with water levels rising and falling 2 to 2.5 ft during our trapping session (Fig. 2.5). No published surveys of freshwater turtle surveys have been completed along the Jourdan River, or in the neighboring Wolf River, so very little is known about the species or communities which reside there.

A total of 23 individuals from 5 species were captured within a single trap session (Table 2.3), that totaled 66 trap nights. CPUE for the Jourdan River was 0.35 per night. This was lower than the Pascagoula River ($\bar{x}_{\text{CPUE}}$ = 0.899), the Big Black River ($\bar{x}_{\text{CPUE}}$ = 0.800), and the Pearl River ($\bar{x}_{\text{CPUE}}$ = 0.433). We believe this is due to the tidal fluctuations which, during low tide, most likely caused the majority of our smaller diameter (3ft) traps to be ineffective for a number of hours. Overall, we captured 8 *M. temminckii* (RA = 0.35), 7 *S. carinatus* (RA = 0.30), 5 *A. spinifera* (RA = 0.22), 2 *P. concinna* (RA = 0.09), and a single Pond Slider (RA = 0.04). Likewise, the only species observed basking were *A. spinifera* (Individuals basking/ river km = 0.268), *P. concinna* (Individuals basking/ river km = 2.33), and *T. scripta* (Individuals basking/ river km = 0.178).

As there has been practically no freshwater turtle research completed along the stretches of the Jourdan River, we were very interested in the possibility of a map turtle species presence, possibly *G. Pearlensis* or *G. oculifera*, that had migrated from the nearby Pearl River Drainage. However, during our survey we did not observe or capture any *Graptemys* species. As species within this genus are known to be prolific baskers (Boyer 1965; Ernst et al. 1994), the complete lack of basking individuals has led us to believe that no *Graptemys* species are present within our surveyed stretch of the Jourdan River.

Again, very little work has been done on the turtle species and communities of the Jourdan River. Our single survey has allowed us to establish at least an idea of the five species which dominate the turtle communities there. However, more work is needed to get a better overarching picture of the tributary as a whole. More trap nights are needed to capture species, like *A. mutica* or *C. serpentina*, which are most likely present but tend to have a very low CPUE. Likewise, while we believe there are no map turtles present at our site, more sites, or basking surveys, are needed to be sure that no *Graptemys* species are present in the higher or lower reaches.

2.3.2 Riverine Species Description

2.3.2.1 Family Chelydridae

2.3.2.1.1 Chelydra serpentina

Of the over 1200 turtles that were captured only six of those individuals were *C. serpentina*. Five individuals were captured at sites along the Pearl River (Table 2.3), and one was captured at Pascagoula Site 4 along the Leaf River (Table 2.2). *Chelydra serpentina* had a 1:1 sex ratio overall with three males and three females, all were sexually mature adults according to Ernst and Lovich (2009), and qualitatively males ($\bar{x}_{CL} =$ 26.5 cm, $\bar{x}_{Mass} = 5{,}016.7\ g$) were on average smaller than females ($\bar{x}_{CL} =$ 29.6 cm, $\bar{x}_{Mass} =$ 6,516.7 g). For a species that is generally thought to be common, the relative abundance of *C. serpentina* was extremely low within both the Pascagoula River (RA = 0.002) and the Pearl River (RA = 0.0129), and nonexistent within the Big Black

River or Jourdan River. Generally, the habitats where *C. serpentina* was captured were smaller, slough like habitats, with 83.3% of individuals captured at designated lake sites. These generally small, lentic trap sites, tended to have fewer or no *M. temminckii* in the direct vicinity, leading us to believe these two closely related species are most likely in direct competition with each other. Perhaps the much larger *M. temminckii* exclude *C. serpentina* from the larger Lake and River sites, leading to their capture in much smaller streams and sloughs that we are generally unable to trap during our surveying efforts. The fact these turtles were found in these small slow-moving headwaters, sloughs, and oxbows, probably also attributed to their high leech presence (67.7%) which was higher than any other species.

While the relative abundance of *C. serpentina* was extremely low for the entirety of our survey (RA = 0.005). The species ranges throughout the state (Rhodin, et al., 2017). Therefore, we believe our site selection was not conducive to capturing this species, and their relative abundance is likely much higher within more appropriate habitats across the state as a whole. Surveys of smaller, more seasonal lentic habitats, as well as smaller lotic habitats, would give researchers a much better understanding of the distribution and abundance of this species. Likewise, better understanding the distribution of *C. serpentina* could lend more credibility to the hypothesis of exclusion due to competition.

2.3.2.1.2 Macrochelys temminckii

It is important to mention this community study was a part of survey efforts specifically targeting *M. temminckii*, which could have had an impact the number of individuals captured. That being said, *M. temminckii* was one of the most ubiquitous species caught during our surveys, with species presence recorded at every single trapping location except Pearl Site 5 (Table 2.2, 2.3, & 2.4). A total of 273 individuals were caught, with significantly more individuals caught per day on the Big Black River ($\bar{x} = 6.78, \chi^2 = 8.42, DF = 2, p = 0.0149$), compared to both the Pascagoula River ($\bar{x}$ = 2.21 individuals per day) and the Pearl River ($\bar{x} = 2.42$ individuals per day). Overall this species had a sex ratio that did not significantly differ from 1:1 (χ^2 = 0.043, p = 0.8448), with a total of 47 females, and 45 males. However, the large majority of these females were caught in the Pascagoula River, which had a sex ratio closer to 2 females:1 male (χ^2 = 6.811, p = 0.00906). The 180 remaining individuals were all classified as juveniles (Ernst & Lovich, 2009).

The Big Black River in particular was inundated with juvenile *M. temminckii*, 206 trap nights yielded a total of 64 individuals, 50 or 79.4% of which were juveniles. Likewise, at the Big Black River sites ($\bar{x} = 16.67, SD = 9.29$) we caught on average significantly more ($F_{2,24}$ = 5.59, p = 0.0102) juvenile individuals than on both the Pearl River ($\bar{x} = 5.38, SD = 2.29$) and the Pascagoula River ($\bar{x} = 5.27, SD = 3.41$). This is interesting, as when we look at similar comparisons of females ($\chi^2 = 3.34, df = 2, p = 0.1865$) and males ($\chi^2 = 1.41, df = 2, p = 0.4930$) there is no significant difference in numbers caught between systems. The Big Black River will be surveyed further in the upcoming 2019 season, and additional trap sites will hopefully yield a clearer picture of the juvenile abundances throughout the system.

Due to the generalist and competitive nature of this species, they are able to successfully occupy a wide variety of habitats, and our surveys caught individuals in both lake and riverine environments. And while we saw no difference in capture rates (t =

$1.8378, df = 25, p = 0.0780$) between lakes ($\bar{x}_{individuals\ per\ site} = 6, SD = 4.34, N = 4$) and rivers ($\bar{x}_{individuals\ per\ site} = 10.55, SD = 6.68, N = 20$), we did see differences in morphology. The carapace length (CL) of both lake males ($\bar{x} = 47.57\ cm, SD = 6.69, N = 11$) and lake females ($\bar{x} = 42.06\ cm, SD = 2.72, N = 19$) are significantly larger (♂ $t = -2.59, p = 0.0314, N = 39$; ♀ $t = -2.22, p = 0.0315, N = 44$) than that of river males ($\bar{x} = 42.29\ cm, SD = 5.44, N = 30$) and females ($\bar{x} = 39.07\ cm, SD = 3.16, N = 29$). The reason for this size discrepancy is not yet known, but could be due to a number of causes, from resource availability to the species' territorial nature. Further lake and river sites will have to be surveyed, to observe if this pattern persists.

Macrochelys temminckii rarely bask, and seldom leave the safety of the water. This behavior most likely lends itself to the high leech presence found in this species compared to some of the other commonly caught species. 53.61% of individuals had some sort of parasite load. Leeches were more likely to be present in the 20.1% of individuals that possessed some sort of injury ($\chi^2 = 7.694, p = 0.0055$). Anecdotally, six *M. temminckii* were observed basking. We were able to hand capture two basking juveniles, both of which had leeches present. There is a possibility that at least juveniles of the species bask more frequently than previously thought in structures such as large root masses which provide more camouflage and cover, to reduce ectoparasitic load or improve health (McAuliffe, 1977).

Overall the relative abundance of *M. temminckii* was surprisingly high, as they were ranked the second most abundant species in the Pascagoula River Drainage (RA = 0.164) (Table 2.7) and the Pearl River Drainage (RA = 0.245) (Table 2.10) and the most abundant species in the Big Black River (RA = 0.388) (Table 2.13) and the Jourdan River site (RA = 0.348). Given the recent commercial harvest, and continued recreational harvest of this species, this type of abundance was unexpected. However, further survey efforts are needed to accurately gauge species distribution and abundance across the state. And future survey efforts are needed to track changes and trends in these initially surveyed populations.

2.3.2.2 Family Emydidae

2.3.2.2.1 Chrysemys picta dorsalis

Although *C. p. dorsalis* can be found throughout much of Mississippi, and has been documented in every drainage, we were able to capture only a single individual at Pearl Site 1 site in the headwaters of the Pearl River (Table 2.8). This juvenile (CL = 3.1 cm, M ~ 5g) was observed basking on a floating debris pile located near the center of the extremely thin stretch of the Pearl River, and was first photographed for documentation and then hand captured.

This species generally prefers slow-moving shallow-water habitats, specifically those that possess ample aquatic vegetation (Ernst & Lovich, 2009). Adjacent to this particular stretch of river were numerous swamp and lake habitats, that are much more stereotypical for *C. p. dorsalis*. There is a good chance this juvenile turtle originated in one of those locations, and was simply washed into the river during a flood event. Surveys of these upland swamps would be needed to fully support these claims. And more extensive surveys of these habitats are needed to better understand the range and abundance of *C. p. dorsalis* throughout Mississippi.

2.3.2.2.2 Graptemys flavimaculata

Graptemys flavimaculata was present at every river site along the Pascagoula River, and individuals were even recorded at Pascagoula lake Sites 11 and 12 (Table 2.5). This is not surprising as their range spans the entirety of the system (Rhodin, et al., 2017), and individuals most likely use these lakes as refugia during flooding events (Jones, 1996). The number of observed individuals ranged from two or three individuals at Pascagoula Site 2, to more than 30 at Pascagoula Site 6. It’s expected that more individuals would be present in the lower stretches of the Leaf or Pascagoula River, as this species tends to prefer the larger sections of the system (Selman & Lindeman, 2015; Lindeman, 1998).

Although we observed numerous individuals, we had only a single capture, at Pascagoula Site 6 site (Table 2.2). This site had by far the largest population, based on our basking survey, and this individual was most likely an accidental catch, either getting caught after trying to bask or simply wandering in. We speculate this, as the species is not known to be piscivorous, their diet generally consists of freshwater sponges (Shelby & Mendonca, 2001), and it was the only individual caught in over 756 trap nights.

2.3.2.2.3 Graptemys gibbonsi

Graptemys gibbonsi, like *G. flavimaculata*, was present at every river site within the Pascagoula River drainage, and recorded at Pascagoula Sites 11 and 12 (Table 2.5). Again, individuals most likely use these lakes as refugia during flooding events and become trapped when the high waters recede. Unlike the *G. flavimaculata* however, *G. gibbonsi* was also captured at every river site, with a total of 31 individuals caught (3 hand captured) (Table 2.2). Capture rates ranged from two individuals caught at

Pascagoula Sites 2 and 8 (CPUE = 0.021), up to 10 individuals at the Pascagoula Site 7 (CPUE = 0.204). The sex ratio differed significantly from 1:1 ($\chi^2 = 9.941, df = 1, p = 0.0016$), with a total of 15 females, and only two males. A total of fourteen individuals were designated as sexually immature juveniles according to Ernst and Lovich (2009).

Graptemys gibbonsi showed relatively low instances of injury (12.9% of individuals), half of these injuries could be linked to predation. A female captured at Pascagoula Site 6 was missing the tip of her tail, this injury is frequent in *M. temminckii* which will cannibalize each other or could reflect other aggressive interactions. A small male from the Pascagoula Site 7 site had a triangular bite mark; this individual was collected and taken to the Central Mississippi Turtle rescue. While there it was discovered the bite had punctured his lung, and he died not soon after. These two injuries, especially the triangular bite, points to the possibility of *M. temminckii* feeding on Map turtles.

Graptemys gibbonsi likewise showed very little leech presence, which could be due to the Map turtle's propensity for basking behavior. On average there was 2.00 *G. gibbonsi* basking per river kilometer. And this ranges from 0.218 individuals per river kilometer at Pascagoula Site 2, to 3.04 individuals per river kilometer at Pascagoula Site 6. *G. gibbonsi* were more abundant baskers than *G. flavimaculata* at every river site surveyed.

2.3.2.2.4 Graptemys oculifera

Graptemys oculifera is a species endemic to the Pearl River, and as such is located within the state of Mississippi and Louisiana. As the sister species to *G. flavimaculata* (Lamb et al. 1994; Stephens and Wiens 2003) of the Pascagoula River, *G.*

oculifera in all probabilities has a diet that that similarly specializes in freshwater sponges (Selman & Lindeman, 2018), and as such the species was rarely captured in our traps. We did, however, catch one female at Pearl Site 2 (Table 2.3), like the single *G. flavimaculata* this was most likely due to chance and not because the individual was attracted to the bait.

All together we captured seven individuals, the single female captured in our traps as mentioned above, as well as a single male and five juveniles which were all hand captured. The species was present at every site (Table 2.8), although had much lower densities at lake sites. We were able to hand capture a larger number of *G. oculifera* compared to *G. flavimaculata* both due to an excess of time as we caught significantly less turtles in the Pearl River, and due to the higher abundances present. This is likely in part due to our ability to trap in sites on lower stretches of the Pearl River, unlike the Pascagoula River trapping season in which we were reduced to trapping numerous lake sites due to flooding and were unable to trap any Pascagoula River mainstem river stretches.

Pearl Site 3 in particular possessed a basking abundance ($\bar{x} = 23.3$ individuals per river km) of *G. oculifera* that far outweighed any other species in the entirety of our study. The average number of individuals observed basking at this site was 95.7 individuals, with a maximum count of 114 individuals (27.8 individuals per river km). And it can be assumed that, with the methods of our basking survey, numerous individuals are missed due to position or bailing from basking spot and our count is therefore an underestimation.

2.3.2.2.5 Graptemys ouachitensis

Graptemys ouachitensis ranges throughout much of the Mississippi River drainage basin (Rhodin et al., 2017). The state of Mississippi likewise has very few county records straying far from the Mississippi River. As such, no counties bordering the Big Black River other than Warren, and Claiborne, both located along the Mississippi River, have any known historical records of *G. ouachitensis.* However, we were able to capture a single individual at Big Black Site 3, which lies between Yazoo, and Madison counties (Table 2.4).

This particular individual was an adult female (CL = 17.7 cm, PL = 15.6 cm, M = 700 g), captured in a trap baited with carp. *G. ouachitensis* is known to readily exploit food resources, and come to baited traps (Vogt, 1981). With this in mind, the fact that we were only able to capture a single individual may point to how small of a population resides there. This is even more so when compared to *G. pseudogeographica,* a similarly widespread species which also inhabits in the Big Black River, and for which we captured 20 individuals. This, as well as the overall inaccessibility of the Big Black River, makes the lack of historical records understandable. And more surveys are necessary to determine the range and extent of *G. ouachitensis* within the Big Black River. But at this time, we believe there is probably a reproducing population present there.

2.3.2.2.6 Graptemys Pearlensis

Graptemys Pearlensis, a species endemic to the Pearl River drainage, was present at eight out of ten river sites, and one lake site (Table 2.8). We captured a total of 27 individuals (Table 2.3), fewer than the closely related *G. gibbonsi*, of which we captured

33. Catch rates ranged from 9 individuals at Pearl Site 1, (CPUE = 0.136) to 1 individual at Pearl Sites 7 (CPUE = 0.015) and 12 (CPUE = 0.02). A total of 14 adults were captured, 8 females and 6 males, the sex ratio was not significantly different from 1:1 ($\chi^2 = 0.286, df = 1, p = 0.593$). A total of 13 captured individuals were sexually immature according to Ernst and Lovich (2009), and all were hand captured. Likewise, three of the six male individuals were hand captured.

Graptemys Pearlensis showed the lowest instances of injury (7.4% of individuals) among all species, with one female that was blind in her left eye, and a male that had old injuries to both his front feet. They likewise have low instances of leech presence (14.8%). However, all individuals that had leeches present were females, and therefore 50% of females possessed some sort of ectoparasite. The general lack of leeches in juveniles and males could be due to the Map turtle's propensity for basking, and the fact that females are much more likely to aquatic bask (Bulté, et al., 2010), compared to juveniles or males, therefore allowing the parasites to remain attached. On average there was 1.86 *G. Pearl Riverensis* basking per river kilometer, this was much less than *G. oculifera,* of which there was an average of 6.25 individuals per river kilometer.

2.3.2.2.7 Graptemys pseudogeographica

Like *G. ouachitensis, G. pseudogeographica* ranges throughout most of the Mississippi River drainage basin. However, this species consists of two subspecies, the False Map turtle (*G. p. pseudogeographica*) which occupies a more northern range (North Dakota, South Dakota, Nebraska, Minnesota, Wisconsin, Iowa, and Illinois), and the Mississippi Map turtle (*G. p. kohnii)* which can be found throughout much of the Central South (Oklahoma, Arkansas, Tennessee, Texas, Louisiana, Mississippi, and

Alabama), with a large intergrade area in between (Illinois, Missouri, Kansas, and Virginia). However, we captured individuals that possessed the key characteristics of both subspecies at two of our sites along the Big Black River, therefore the intergradation zone must stretch farther south than previously thought (Table 2.4).

Like most *Graptemys* species, females ($\bar{x}_{\mathrm{CL}}$ = 18.6 cm, $\bar{x}_{\mathrm{mass}}$ = 887.2 g) were larger than males ($\bar{x}_{\mathrm{CL}}$ = 10.95 cm, $\bar{x}_{\mathrm{mass}}$ = 120 g). We captured a total of 14 females, 5 males, and one juvenile, which differs significantly from a 1:1 sex ratio (χ^2 = 4.26, df = 1, p = 0.03895). However, due to our low number of captures there is a high chance this sex ratio does not accurately describe the population. Individuals that presented as *kohnii* ($\bar{x}_{\mathrm{CL}♀}$ = 17.95 cm, $\bar{x}_{\mathrm{CL}♂}$ = 10.74 cm) were on average smaller than those that presented as *pseudogeographica* or intergrades ($\bar{x}_{\mathrm{CL}♀}$ = 19.78 cm, CL♂ = 11.8 cm). However, in females this difference was not significant (*t* = 1.141, df = 12, p = 0.2762).

The majority of *G. pseudogeographica* were captured at Big Black Site 3 (10 individuals, 50% of all *G. pseudogeographica* captured, CPUE = 0.145). At this site the overall relative abundance of *G. pseudogeographica* was 14% of the total turtle captures, with 7 individuals which presented distinct *G. p. kohnii* features (70%) and 3 individuals that presented distinct *G. p. pseudogeographica* features (30%). This pattern continued at Big Black Site 2, where 8 individuals were captured (RA = 0.20, CPUE = 0.118), 5 of which presented *G. p. kohnii* features (62.5%), the remaining 3 individuals presenting more so as *G. p. pseudogeographica* (37.5%). Big Black Site 1 differed however, as we only captured two individuals which both presented *G. p. kohnii* patterning (RA = 0.05, CPUE = 0.029) (Table 2.13).

Overall it makes sense that the majority of individuals captured (70%) had the distinct traits of *G. p. kohnii*, as they have been recorded to range into the upper stretches of the Big Black River (Rhodin, 2017). The intergrade area, let alone the range of the *G. p. pseudogeographica* was thought to be much farther north, around the borders of Arkansas, Tennessee and Missouri. However, we did catch numerous individuals that presented as intergrades, with more *G. p. pseudogeographica* features. Therefore, more surveys, and possibly genetic studies should be completed to fully understand the genetics and distribution of this species, within the stretches of the Big Black River drainage.

2.3.2.2.8 Pseudemys concinna

Pseudemys concinna is a widespread species, and can be found in 19 Southern states, from northern Virginia south to Florida, and throughout the Gulf Coastal Plain to Texas and Kansas. It was widespread in our surveys as well (Table 2.2, 2.3, & 2,4), as we caught a total of 68 individuals, with at least one individual within every drainage sampled. Twenty-two of these individuals were female, and 26 were male; this was not significantly different than a 1:1 sex ratio ($\chi^2 = 0.333$, p = 0.5637). The remaining 20 individuals were all classified as juveniles. Our catch per unit effort (CPUE) in the Pascagoula River ($\bar{x}$ = 0.0876 *P. concinna*/ trap night, SD = 0.068, N = 8), where we caught 45 individuals in 756 trap nights, was higher than that of the Pearl River ($\bar{x}$ = 0.0137 *P. concinna*/ trap night, SD = 0.0138, N =10) in which our total captures was 12 in 870 trap nights. The abundances in the Jourdan River were slightly better with two individuals captured in 66 trap nights (CPUE = 0.03 *P. concinna*/ trap night). However, in the Big Black River we caught a single individual at the Big Black Site 2 (Table 2.4) leading to an exceedingly low relative abundance and catch rate (RA = 0.006, CPUE = 0.005 *P. concinna*/ trap night). Out of the 16 species captured *P. concinna*, had the 5th most captures.

Like their *Emydid* cousins, the Map turtles, *P. concinna* were frequently seen basking in every system other than the Big Black River, in which no individuals were observed. With an average of 0.732 individuals basking per river km within the Pascagoula River drainage, 0.746 individuals basking per river km within the Pearl River, and 2.33 individuals basking per river km within the Jourdan River.

Pseudemys concinna showed a relatively average presence of injuries (20.5% of individuals possessed an injury) when compared to other species, with a total of 13 individuals possessing any sort of injury. The majority of injuries were missing appendages or feet (6 individuals), or aged injuries to the carapace or plastron (8 individuals). *Pseudemys concinna* showed significantly lower ectoparasites (5.26%) when compared to other species ($\chi^2 = 85.11$, $p < 0.0001$). This could be due to basking frequency, similar to the Map turtles, or possibly habitat occupancy. *Pseudemys concinna* tend to occupy spaces of vegetation, or near the surface, this is in stark contrast to the bottom walkers like *M. temminckii* or *S. carinatus*, which both showed the highest leech presence.

2.3.2.2.9 Trachemys scripta

By far the most ubiquitous turtle in the world, and the state of Mississippi, *T. scripta* was captured in all systems, with a total of 485 individuals. Likewise, the species was captured at all but 5 sites (Table 2.2, 2.3, & 2.4). However, individuals were observed basking at Pearl Sites 3 and 4, and Pascagoula Site 10 (Table 2.5 & 2.8), so they are still present in those locations. Overall, we captured 230 females, and 205 males, which did not differ significantly from a 1:1 sex ratio ($\chi^2 = 1.437, df = 1, p = 0.2307$). The other 50 individuals were deemed sexually immature according to Ernst and Lovich (2009).

We caught significantly more (t = -3.698, df = 22, p = 0.0013) individuals at lake sites ($\bar{x}$ = 37.4, SD = 40.55, N = 9) compared to river sites ($\bar{x}$ = 9.375, SD = 13.41, N = 16), with a total of 337 individuals captured in lakes (CPUE = 0.555) and only 151 individuals captured in rivers (CPUE = 0.117). If we look at just river sites across systems, there was no significant difference ($F_{2,13}$ = 0.4604, p = 0.6409) in the number of *T. scripta* caught on Pascagoula River ($\bar{x}$ = 6.14, SD = 8.84, N = 6) river sites, Pearl Sites ($\bar{x}$ = 13.14, SD = 18.55, N = 7), or along the Big Black River ($\bar{x}$ = 7, SD = 5.57, N = 3).

During our surveys we observed two recognized subspecies of Pond Slider, the Red-eared Pond Slider (*T. s. elegans)* and the Yellow-bellied Pond Slider (*T. s. scripta*), as well as obvious intergrades. The majority of individuals that showed *T. s. scripta* or intergrade patterning were within the Pascagoula River drainage, especially at some of our more southern lake sites. This is to be expected as the intergrade range occurs around the edge of the Mississippi - Alabama border (Rhodin, et al., 2017). However, intergrades were also present within the Pearl River drainage, which should generally only be *T. s. elegans*.

Trachemys scripta showed relatively low instances of injury (10.7% of individuals). Of the 52 individuals that had injuries, the majority were injuries to the scutes of the carapace or plastron (47%), injuries to the scutes can be a result of falling, boat/ human interaction, or failed predation attempts from other animals (Vella, 2009), and are quite common across turtle species. Twenty-two percent of injuries were missing appendages (feet, legs, toes, or tail), again presumably due to a failed predation attempt. Likewise, 22% of injuries were what we called “pitting”, which could be described as small circular holes around 1-3 mm deep in the carapace or plastron. These individuals had more extreme forms of pitting, and we did not count individuals with only one or two pit holes. Pitting is thought to either be due to a bacterial or fungal infection of the shell (Carpenter, 1956), or from a withdrawal of both calcium and phosphate from the shell especially for egg production and laying in females (Ernst, 1971). Like Ernst, we did see extreme pitting in more females (9 individuals) compared to males (2 individuals), however our numbers are too low to determine any significance. Pitting is also a signature of shell disease (Hernandez-Divers, et al., 2009), which can be caused by both malnutrition (a lack of calcium), and a variety of fungal or bacterial infections, so both reasons remain possible and may change depending on the individual.

Trachemys scripta likewise showed very little leech presence (13.53% of individuals had ectoparasites), which could be due to their being significantly less ($\chi^2 = 58.97,\ p < 0.0001$) leech presence on turtles captured in lake sites compared to river sites. As previously stated, we captured a significant majority of *T. scripta* within lakes compared to rivers, this could attribute to the overall low rate of leech presence, as 11% of individuals captured on rivers had leeches, compared to only 5% on lakes.

2.3.2.3 Family Kinosternidae

2.3.2.3.1 Sternotherus carinatus

Compared to the other species of musk turtle present in Mississippi, *S. carinatus* was by far the most abundant in larger habitats, like those we surveyed. The species was recorded from all but 7 sites from the Pascagoula River, Pearl River, and Jourdan River (Table 2.2 & 2.3). However, no individuals were captured or observed at our 3 Big Black River sites (Table 2.11). Overall, we captured 23 females, and 53 males, which differed significantly from a 1:1 sex ratio ($\chi^2 = 11.84, df = 1, p = 0.00058$). The Pearl River captures likewise differed significantly from 1:1 ($\chi^2 = 7.41, df = 1, p = 0.0065$) with 11 females, and 28 males. This was not the norm across all systems, as in the Pascagoula River we captured 13 females, and 22 males, which did not differ significantly from 1:1 ($\chi^2 = 2.314, df = 1, p = 0.128$). Whether this skew accurately reflects populations, or is due to a higher mobility, larger size, or willingness of males to come to traps is unknown. The remaining 11 individuals were deemed sexually immature according to Ernst and Lovich (2009).

There was no significant difference in the number of individuals (t = 0.866, df = 18, p = 0.3981) or the catch per unit effort (t = 0.602, df = 18, p = 0.5546) between lake sites ($\bar{x}_{\text{individuals}}$ = 3.2, SD = 1.48, N = 5; $\bar{x}_{\text{CPUE}}$ = 0.05, SD = 0.027, N = 5) when compared to river sites ($\bar{x}_{\text{individuals}}$ = 4.47, SD = 1.11, N = 15; $\bar{x}_{\text{CPUE}}$ = 0.06, SD = 0.04, N = 15). When just river sites are included, there was likewise no significant difference (t = -0.3265, df = 17, p = 0.7408) in the number of *S. carinatus* caught on Pascagoula River ($\bar{x}$ = 4.125 SD = 2.47, N = 8) when compared to the Pearl River ($\bar{x}$ = 3.91, SD = 3.14, N = 11).

Sternotherus carinatus showed relatively higher instances of injury (32.18% of individuals) compared to other species. Of the 31 individuals that had injuries, the majority were male (74% of males captured had injuries), and most of the injuries were to the individuals marginal scutes. Like previously stated a fall can break turtle scutes

(Vella, 2009), and *S. carinatus* are known to be somewhat arboreal in their basking behavior. But *S. carinatus* also are known for male to male combat, and competition for mates (Kavanagh, 2016). It is likely males were more prone to injuries because of their aggressive and violent competition, this is reminiscent of *M. temminckii*, in which males (31%) also had an increased percentage of injuries when compared to females (11%).

Sternotherus carinatus showed a very high leech presence (50.98% of individuals had ectoparasites), second only to *M. temminckii* (53.61%). It is possible this is due to the microhabitats in which the two species occupy. *S. carinatus*, are known as “bottom walkers”, and spend much of their time in direct contact with the substrate, where the leeches reside. Our study, like others, found that these species tend to have a higher parasitic load than those that bask frequently or less frequently come into contact with the substrate (Readel, Phillips, and Wetzel, 2008).

It is still unknown if *S. carinatus* are present within the Big Black River. A previous record lower in the drainage does exist, but the validity of the record location is highly questionable. Continued survey efforts will move down river in the upcoming field season. A larger amount of trap nights and surveys, should allow us to determine if *S. carinatus* are present or absent within the drainage.

2.3.2.3.2 Sternotherus odoratus

Similar to *C. serpentina*, only nine *S. odoratus* were captured, seven were captured within the Pascagoula River Drainage (Table 2.2), and two were captured in the Pearl River Drainage (Table 2.3). We also saw a single individual basking at Pearl Site 1 site within the Pearl River drainage (Table 2.8), but were unable to capture it.

Sternotherus odoratus had a 1.25:1 sex ratio overall with five females and four males, although with so few individuals captured this is obviously not representative of any populations. Males ($\bar{x}_{CL} =$ 7.86 cm, $\bar{x}_{Mass} = 112.5\ g$) were on average smaller than females ($\bar{x}_{CL} =$ 8.28 cm, $\bar{x}_{Mass} =$ 85 g), however they tended to weigh more. For a species that is generally thought to be both common and wide ranging, the relative abundance of *S. odoratus* was extremely low within both the Pascagoula River (RA = 0.008) and the Pearl River (RA = 0.0026), and nonexistent within the Big Black River or Jourdan River. Generally, the habitats where *S. odoratus* was captured were lentic (78%), and the river sites where our two individuals were captured were relatively small ($\bar{x}_{Width\ of\ Upper\ Bouie} =$ 46.1 m, Site on Strong = 28.2 m). These generally small, lentic trap sites, tended to have adjacent swamps with ample cover, which could possibly shelter this small species from potential predators.

While the relative abundance of *S. odoratus* was extremely low for the entirety of our survey (RA = 0.005), much like the Eastern Snapping turtle, the species ranges across the state (Rhodin, 2017). Therefore, we believe our site selection was not conducive to capturing this species, and their relative abundance is most likely much higher within more appropriate habitats across the state. Surveys of smaller lentic habitats, as well as smaller lotic habitats, would give researchers a much better understanding of the distribution and abundance of this species.

2.3.2.3.3 Sternotherus peltifer

Sternotherus peltifer, previously a subspecies of the Loggerhead musk turtle (*S. minor*), was recently elevated to full species status (Scott, et al., 2018). This split, as well as the few closely related, morphologically similar, musk turtles whose ranges intermingle with *S. peltifer* has left its true extent, especially through Mississippi, a bit of a mystery. However, the species is suspected to range from the Pearl River drainage of Mississippi east throughout much of Alabama (Rhodin, et al., 2017). While *S. peltifer* can certainly be found throughout the Pascagoula River drainage (G. Brown, *pers. comm.*), and we have a few accurate historical records from the Southern Pearl River and throughout the Tombigbee, records through the Pearl River drainage are overall lacking. Likewise, during our survey no individuals were captured in the Pearl River, with a total of only two individuals captured, a male (CL = 8.1 cm, PL = 5.3 cm, M = 90 g) and a female (CL = 8.8 cm, PL = 6.4, M = 120 g) at Pascagoula Site 2 (Table 2.2 & 2.5).

While a number of individuals have been captured throughout the Pascagoula River Drainage, the majority of both recent (G. Brown, *pers. comm.*) and historical records were captured in small streams and creeks, as opposed to the larger river systems we surveyed. The species is known to prefer smaller lotic habitat, with gravel or stone substrate, and clear water. Therefore, the majority of our survey sites were not in locations conducive to capturing *S. peltifer*. However, this alone does not explain the lack of captures at Pascagoula Site 7 site in particular, which has numerous recent and historical records. At the time of our survey of Pascagoula Site 7, from July 15th through July 18th, water discharge levels ($\bar{x}$ = 1,290^{3}ft/s) were twice their normal July level ($\bar{x}$ = 610^{3}ft/s) (USGS, Hydrologic Unit 03170002). While we did capture two closely related male *S. carinatus*, this flooding could have prevented *S. peltifer* from entering our traps. However, male *S. carinatus* are slightly larger than Stipe-necked musk turtles. Therefore, it is possible, that the smaller *S. peltifer,* much like *S. odoratus,* are for some reason excluded from the larger traps we used during our surveys. A study on trap efficiency, as it pertains to catch per unit effort for *S. peltifer*, would have to be completed to be certain.

2.3.2.4 Family Trionychidae

2.3.2.4.1 Apalone mutica

Unlike the closely related *A. spinifera*, *A. mutica* were rarely captured. A total of only 28 individuals were caught, with significantly more individuals caught per day (t = -4.49, df = 6, p = 0.0041) on the Big Black River ($\bar{x} = 6.33, SD = 2.08, DF = 2, N = 3, 19\text{ individuals}$) (Table 2.4), than on the Pascagoula River ($\bar{x}$ = 1.8, SD = 0.837, N = 5, 9 individuals) (Table 2.2), while no individuals were captured at any sites along the Pearl River or on the Jourdan River. We captured individuals representing both subspecies, with those captured within the Pascagoula River Drainage representing the Gulf Coast Smooth Softshell (*A. m. calvata)*, and those captured in the Big Black River representing the Midland Smooth Softshell turtle (*A. m. mutica)*. Females of both subspecies resembled each other closely, with females of the Gulf Coast subspecies, showing a slightly more pronounced pattern. Males had distinctly different patterns, the carapace of Midland individuals had a spattering of small (2-3 mm) oval or circular dots and a bright posterior ocular line, while Gulf Coast individuals had much larger spots on the carapace (2-3 cm), and a post ocular line that possessed a more yellow coloration.

The sex ratio of this species was significantly different from 1:1 (χ^2 = 7.348, df = 1, p = 0.0067), with a total of 18 females captured, compared to only 5 males. It is possible, due to our small sample size, that these number do not accurately reflect the population. However, *A. spinifera* likewise show a sex ratio that is significantly different from 1:1 (χ^2 = 85.54, df = 1, p <0.0001). Therefore, either males are extremely unlikely to enter traps compared to females, or the populations are dominated by females. It is entirely possible that the overall population of softshells are mostly females, due to their extreme sexual dimorphism. Females can grow much larger than males (SCL_{Max}♂ = 26.6 cm, SCL_{Max}♀ = 35.6 cm, Ernst & Lovich, 2009), this may reduce overall predation pressure, and increase female survivorship.

A. mutica had a presence of ectoparasites in 38.9% of individuals. This was higher than all other species, except for the bottom walkers (*M. temminckii* and *S. carinatus*). This is most likely due to the burrowing behavior that softshell turtles exhibit to either avoid danger, or as a method of ambush hunting. Injuries rates were the highest among *A. mutica*, with 44.4% of individuals presenting some form of injury. The large proportion of these injuries were bites, marks, or holes to the carapace (75%), which due to its comparatively soft nature, in contrast to a keratinized turtle shell, is much easier to puncture or scratch.

Overall, *A. mutica* had an extremely low capture rate ($\bar{x}_{CPUE}$ = 0.023), with only about a 2.3% chance of capturing an individual in a trap night. This was slightly higher on the Big Black River ($\bar{x}_{CPUE}$ = 0.092) where there was around a 9.2% chance of capturing a single individual in a single trap night, compared to the Pascagoula River ($\bar{x}_{CPUE} = 0.012$) in which there was only a 1.2% chance. And while CPUE seems to be low overall for the species, their relative abundance was much greater on the Big Black River ($\bar{x}_{RA}$ = 0.119) (Table 2.13) compared to the Pascagoula River ($\bar{x}_{RA}$ = 0.038) (Table 2.7). Meaning while there is still a low chance of capturing an individual, they make much more of the overall community of the Big Black River.

Finally, the complete lack of captures along the Pearl River was surprising, as there are numerous historical records throughout the lower Pearl River. One individual was observed basking at Pearl Site 10. Therefore, *A. mutica* are present within the Pearl River, however it is possible they are in very low abundances. Surveys of the Pearl sites that take place for longer periods, thus allowing a higher number of trap nights would most likely produce more *A. mutica*, and would be better suited for the study of this species.

2.3.2.4.2 Apalone spinifera

As the third most abundant species (RA = 0.148), *A. spinifera* was wide ranging and plentiful throughout most river sites (Table 2.2, 2.3, & 2.4). A total of 182 individuals were caught, with significantly more individuals caught per day ($F_{2,15}$ = 11.53, p = 0.0009) on the Big Black River ($\bar{x} = 12.67,\ SD = 6.03\ N = 3$) and Pascagoula River ($\bar{x}$ = 14.83, SD = 7.99, N = 6) river sites, compared to the Pearl River ($\bar{x} = 2.89, SD = 2.05, N = 9$). Likewise, CPUE was significantly higher ($F_{2,15}$ = 11.15, p = 0.0011) on the Big Black River ($\bar{x} = 0.184,\ SD = 0.07\ N = 3$) and Pascagoula River ($\bar{x}$ = 0.234, SD = 0.114, N = 6) river sites, compared to the Pearl River ($\bar{x} = 0.045, SD = 0.033, N = 9$).

However, if we look at number of individuals captured per site, or CPUE, when we include lake sites, there is no significant difference (Individuals per site: $F_{2,22}$ = 2.972, p = 0.0712, CPUE: $F_{2,22}$ = 2.884, p = 0.0772) between the Big Black River (Individuals per site: $\bar{x} = 12.67,\ SD = 6.03\ N = 3,$ CPUE: $\bar{x} = 0.184,\ SD = 0.09\ N = 3$), Pascagoula River (Individuals per site: $\bar{x} = 9.6,\ SD = 9.05\ N = 10,$ CPUE: $\bar{x} = 0.15,$ $SD = 0.151\ N = 10$), or Pearl River (Individuals per site: $\bar{x} = 3.58,\ SD = 3.32\ N =$

12, CPUE: $\bar{x} = 0.055,\ SD = 0.05\ N = 12$). But we believe this data is skewed. Our catch per unit effort on Pearl River lake sites (CPUE = 0.09) is higher than rivers (CPUE = 0.05), although this is mainly due to the amount of *A. spinifera* that were captured at Pearl Site 7, a location that has known instances of captive *A. spinifera* release (C. Milbourne, *pers. comm.*). When Pearl Site 7 was removed, we once again saw significantly higher numbers in the Big Black River and Pascagoula River, compared to the Pearl River (Individuals per site: $F_{2,22} = 3.861$, $p = 0.0373$, CPUE: $F_{2,22} = 3.752$, $p = 0.045$).

Like *A. mutica*, the sex ratio of this species was significantly different from 1:1 ($\chi^2 = 85.54$, df = 1, $p < 0.0001$), with a total of 148 females captured, compared to only 26 males. It is possible, that these numbers do not accurately reflect the population, and males are simply extremely unlikely to enter traps compared to females. However, it is again possible that the overall population of softshells are mostly females, due to their extreme sexual dimorphism, which is even more exaggerated than what is seen in *A. mutica*. Females can grow much larger than males, (SCL_{Max}♂ = 21.6 cm, SCL_{Max}♀ = 54.0 cm, Ernst & Lovich, 2009), which may reduce overall predation pressure, and produce populations that are dominated by females.

Apalone spinifera had the presence of ectoparasites in 37.8% of individuals. This was higher than all other species, except for the bottom walkers (*M. temminckii* and *S. carinatus*), and *A. mutica* (38.9%). Like *A. mutica*, this is most likely due to the burrowing behavior that softshell turtles exhibit. Burying themselves beneath the substrate to avoid danger, or as a hiding method for ambush predation. Injury rates of *A. spinifera* (22.04%) were comparable to *M. temminckii* (20.5%) and *P. concinna* (20.5%), with a large proportion of these injuries were bites, marks, or holes on the carapace (65%). At least 3 individuals had signs of injury to the face, neck, and shell that were caused by fishing hooks, including one individual which had the hook lodged in its throat. The hook entered the individual's mouth, and curled to exit the right ventral side of the individuals throat. Similarly, a second individual was caught with healed wounds that mirrored the previously described hook entry/ exit. However, the exit wound on the neck was a much larger opening, and the entry point on the mouth much more scarred. This hook had most likely remained in place for several days to weeks, whereas the previous individual had been hooked, released, and then the hook removed within a few hours (fisherman, *pers. comm*.). Both of these individuals were captured at Pearl Site 7.

Overall, we captured *A. spinifera* from every system, and at 26 of our 30 trap sites. Other than *M. temminckii*, which were captured at 29 of 30 trap sites, *A. spinifera*, was the most widely distributed species. Of the four sites where no individuals were captured, only one was a river site, while the remaining 3 were all lake sites (Table 2.8). Softshell turtles, are generally thought to prefer lotic habitats, which we likewise observed, as we captured significantly more individuals (χ^2 = 5.695, df = 1, p = 0.0170) on river sites ($\bar{x}$ = 8.32, SD = 7.52, N = 19) compared to lakes ($\bar{x}$ = 2, SD = 1.55, N = 6), and had a significantly higher CPUE (χ^2 = 3.895, df = 1, p = 0.0484) on rivers ($\bar{x}$ = 0.128, SD = 0.123, N = 19) compared to lakes ($\bar{x}$ = 0.034, SD = 0.003, N = 6).

Pearl Site 12, the only riverine site in which we did not capture *A. spinifera*, only had a total of 46 trap nights due to weather constraints, compared to the average 62. The average CPUE for *A. spinifera* along our Pearl sites was 0.055, or a 5.5% chance of capturing a single *A. spinifera* in a single trap night. However, in some locations it could be as low as 0.015, or only a 1.5% chance of catching a single individual in a single trap night. At capture rates this low, 46 trap nights may not be enough to catch any individuals. Indeed, *A. spinifera* was captured both upstream and downstream of this site. Therefore, we believe there are *A. spinifera* at this site, however they are present in lower densities.

Overall, this survey has obtained baseline data throughout much of the Pearl River and Pascagoula River Drainages, a small section of the Jourdan River, and has begun a portion of the Big Black River. Lower sections of the Pascagoula River were not surveyed due to weather and time constraints, and should be further surveyed for a better understanding of the species distributions and community make-up of the Lower Leaf, and the Pascagoula River Proper. Surveys will continue across the State to obtain a more encompassing scope of the turtle species abundance and distribution across the entire State of Mississippi.

2.4 Tables

Table 2.1 List of Sites Surveyed.

Pascagoula River Drainage					
ID #	Site:	Trap Ngiths	Latitude	Longitude	Type
1	Murchinson Lake	60	31.4400	-89.4404	Lentic
2	Upper Bouie	95	31.4227	-89.3937	Lotic
3	Upper Leaf	78	31.6888	-89.4030	Lotic
4	Pierce Lake	56	31.3873	-89.2751	Lentic
5	Wedgeworth	49	31.2751	-89.2311	Lentic
6	Middle Leaf	62	31.1952	-88.9248	Lotic
7	Upper Chick	49	32.1123	-88.8081	Lotic
8	Middle Chick	63	31.5179	-88.5420	Lotic
9	Lower Chick	47	31.1816	-88.5915	Lotic
10	Charles Deaton	58	31.0015	-88.7085	Lentic
11	Pascagoula WMA	74	30.9048	-88.7404	Lentic
12	Rhymes Lakes	65	30.8153	-88.7336	Lentic
Pearl River Drainage and Jourdan River					
1	Philadelphia	68	32.8296	-89.1221	Lotic
2	Carthage	66	32.7151	-89.4983	Lotic
3	Coal Bluff	65	32.6058	-89.7640	Lotic
4	Ross Barnett North	58	32.5588	-89.8600	Lentic
5	Ross Barnett South	53	32.3953	-90.0052	Lentic
6	LeFleur's Bluff	68	32.3281	-90.1476	Lentic
7	Crystal Lake	66	32.2940	-90.1572	Lentic
8	Georgetown	68	31.9236	-90.1665	Lotic
9	Atwood	46	31.5830	-90.0891	Lotic
10	Columbia	66	31.3117	-89.8782	Lotic
11	Bogalusa	68	30.7890	-89.8219	Lotic
12	Walkiah Bluff	46	30.6096	-89.8221	Lotic
13	Stennis	64	30.3863	-89.6707	Lotic
14	Bogue Chitto	68	31.1873	-90.2919	Lotic
15	Jourdan River	66	30.4041	-89.4888	Lotic
Big Black River Drainage					
1	Goodman	69	32.9430	-89.8998	Lotic
2	Vaughan	68	32.7204	-90.0838	Lotic
3	Bentonia	69	32.6113	-90.3467	Lotic

Site numbers match those on Figures 2.2 through 2.4.

Table 2.2 Pascagoula River Drainage Captures

Pascagoula River Drainage		Total Trap Captures											Site Captures
ID #	Site:	***A.m.***	***A.s.***	***C.s.***	***G.f.***	***G.g.***	***M.t.***	***P.c.***	***S.c.***	***S.o.***	***S.p.***	***T.s.***	
1	Murchinson Lake	0	1	0	0	0	1	0	0	2	0	45	49
2	Upper Bouie	0	17	0	0	2	8	15	8	1	2	5	58
3	Upper Leaf	2	18	0	0	4	7	1	6	0	0	2	40
4	Pierce Lake	0	0	1	0	0	8	11	3	1	0	136	160
5	Wedgeworth	0	4	0	0	0	4	0	0	0	0	30	38
6	Middle Leaf	1	3	0	0	7	12	3	6	0	0	1	33
7	Upper Chick	3	22	0	0	10	9	7	4	0	0	24	79
8	Middle Chick	2	22	0	1	8	18	3	1	0	0	3	58
9	Lower Chick	1	7	0	0	2	11	3	0	0	0	2	26
10	Charles Deaton	0	1	0	0	0	11	0	4	0	0	0	16
11	Pascagoula WMA	0	1	0	0	0	5	0	0	1	0	21	28
12	Rhymes Lakes	0	0	0	0	0	12	2	1	0	0	46	61
	Sum	9	96	1	1	33	106	45	33	5	2	315	646

Total number of individual turtles captured by species by site in the Pascagoula River drainage.

Table 2.3 Pearl and Jourdan River Drainage Captures

Pearl River Drainage		Total Trap Captures										Site Captures
ID #:	Site:	**A.s.**	**C. p.**	**C.s.**	**G.o.**	**G.p.**	**M.t.**	**P.c.**	**S.c.**	**S.o.**	**T.s.**	
1	Philadelphia	1	1	1	0	9	3	2	1	0	12	30
2	Carthage	5	0	0	3	7	5	0	3	0	2	25
3	Coal Bluff	1	0	0	0	0	7	1	11	0	0	20
4	RB - North	1	0	0	0	0	9	1	5	0	0	16
5	RB - South	0	0	0	0	0	5	0	0	0	4	9
6	LeFleur's Bluff	4	0	4	0	0	0	0	0	0	39	47
7	Crystal Lake	12	0	0	0	0	7	0	3	1	16	39
8	Georgetown	5	0	0	0	1	13	2	4	0	10	35
9	Atwood	2	0	0	0	2	5	1	1	0	0	11
10	Columbia	2	0	0	4	5	6	1	2	0	0	20
11	Bogalusa	2	0	0	0	0	8	3	4	0	54	71
12	Walkiah Bluff	0	0	0	0	1	6	0	1	0	10	18
13	Stennis	7	0	0	0	0	13	1	0	0	1	22
14	Bogue Chitto	1	0	0	0	2	8	3	8	0	3	25
	Sum	43	1	5	7	27	95	15	43	1	151	388
Jourdan River Drainage												
15	Jourdan River	5	0	0	0	0	8	2	7	0	1	23

Total number of individual turtles captured by species by site in the Pearl and Jourdan River drainages.

Table 2.4 Big Black River Drainage Captures

Big Black River Drainage		Total Trap Captures								Site Captures
ID #:	Site:	**A.m.**	**A.s.**	**G.oua.**	**G.p.k.**	**G.p.p.**	**M.t.**	**P.c.**	**T.s.**	
1	Goodman	7	7	0	2	0	13	0	13	42
2	Vaughan	4	12	0	5	3	18	1	2	45
3	Bentonia	8	19	1	8	3	33	0	6	78
	Sum	19	38	1	15	6	64	1	21	165

Total number of individual turtles captured by species by site in the Big Black River drainage.

Table 2.5 Pascagoula River Drainage Species Observed

Pascagoula River Drainage		Species Observed											Total Diversity
ID #	Site:	***A.m***	***A.s***	***C.s***	***G.f***	***G.g***	***M.t***	***P.c***	***S.c***	***S.o***	***S.p***	***T.s***	
1	Murchinson Lake	0	1	0	0	0	1	0	0	1	0	1	4
2	Upper Bouie	0	1	0	1	1	1	1	1	0	1	1	8
3	Upper Leaf	1	1	0	1	1	1	1	1	0	0	1	8
4	Pierce Lake	0	0	1	0	0	1	1	1	1	0	1	6
5	Wedgeworth	0	1	0	0	0	1	0	0	0	0	1	3
6	Middle Leaf	1	1	0	1	1	1	1	1	0	0	1	8
7	Upper Chick	1	1	0	1	1	1	1	1	0	0	1	8
8	Middle Chick	1	1	0	1	1	1	1	1	0	0	1	8
9	Lower Chick	1	1	0	1	1	1	1	0	0	0	1	7
10	Charles Deaton	0	1	0	0	0	1	0	1	0	0	1	4
11	Pascagoula WMA	0	1	0	1	1	1	0	0	0	0	1	3
12	Rhymes Lakes	0	0	0	1	1	1	1	1	1	0	1	5

The species observed at each site within the Pascagoula River drainage, as well as the total species for each site. (0 = species not observed, 1 = species observed)

Table 2.6 Pascagoula River Drainage Species Richness Measurements

ID #:	Site:	Species Richness	D	E_D	H	J
1	Murchinson Lake	4	1.18	0.30	0.37	0.27
2	Upper Bouie	8	4.98	0.62	1.77	0.85
3	Upper Leaf	7	3.69	0.53	1.57	0.81
4	Pierce Lake	6	1.37	0.23	0.61	0.34
5	Wedgeworth	3	1.55	0.52	0.66	0.60
6	Middle Leaf	7	4.37	0.62	1.65	0.85
7	Upper Chick	7	4.75	0.68	1.72	0.88
8	Middle Chick	8	3.75	0.47	1.57	0.75
9	Lower Chick	6	3.60	0.60	1.49	0.83
10	Charles Deaton	3	1.86	0.62	0.78	0.71
11	Pascagoula WMA	4	1.68	0.42	0.76	0.55
12	Rhymes Lakes	4	1.64	0.41	0.71	0.51

The species richness calculations of, Simpsons Index (D), Simpsons Equitability (E_D), Shannon's Diversity (H), and Shannon's Equitability (J) for each site within the Pascagoula River Drainage.

Table 2.7 Pascagoula River Drainage Relative Abundances

Species	Overall	Lakes	Rivers
A. mutica	0.01	0	0.03
A. spinifera	0.15	0.02	0.25
C. serpentina	0.001	0.002	0
G. flavimaculata	0.002	0	0.003
G. gibbonsi	0.05	0	0.09
M. temminckii	0.16	0.12	0.18
P. concinna	0.07	0.04	0.09
S. carinatus	0.05	0.02	0.07
S. odoratus	0.008	0.01	0.003
S. peltifer	0.003	0	0.01
T. scripta	0.49	0.79	0.11

The relative abundance of all species within the Pascagoula River Drainage, overall, and then broken down by total trap nights on lake (lentic) sites, and riverine (lotic) sites.

Table 2.8 Pearl and Jourdan River Drainages Species Observed

Pearl River Drainage		Species Present										Total Diversity
ID	Site:	**A.s**	**C.p**	**C.s**	**G.o**	**G.p**	**M.t**	**P.c**	**S.c**	**S.o**	**T.s**	
1	Philadelphia	1	1	1	1	1	1	1	1	1	1	10
2	Carthage	1	0	0	1	1	1	0	1	0	1	6
3	Coal Bluff	1	0	0	1	1	1	1	1	0	1	7
4	RB - North	1	0	0	1	0	1	1	1	0	1	6
5	RB - South	1	0	0	1	1	1	0	0	0	1	5
6	LeFleur's Bluff	1	0	1	1	0	0	1	0	0	1	5
7	Crystal Lake	1	0	0	1	0	1	0	1	1	1	6
8	Georgetown	1	0	0	1	1	1	1	1	0	1	6
9	Atwood	1	0	0	1	1	1	1	1	0	0	6
10	Columbia	1	0	0	1	1	1	1	1	0	0	7
11	Bogalusa	1	0	0	1	0	1	1	1	0	1	6
12	Walkiah Bluff	0	0	0	1	1	1	1	1	0	1	6
13	Stennis	1	0	0	1	0	1	1	0	0	1	5
14	Bogue Chitto	1	0	0	1	1	1	1	1	0	1	6
Jourdan River Drainage												
15	Jourdan River	1	0	0	0	0	1	1	1	0	1	5

The species observed at each site within the Pearl River drainage and at the Jourdan River site, as well as the total species for each site. (0 = species not observed, 1 = species observed)

Table 2.9 Pearl River Drainage Species Richness Measurements

ID #:	Site:	Species	D	E_D	H	J
1	Philadelphia	8	3.72	0.46	1.59	0.77
2	Carthage	6	5.17	0.86	1.71	0.95
3	Coal Bluff	4	2.33	0.58	1.00	0.72
4	RB - North	4	2.37	0.59	1.03	0.75
5	RB-South	2	1.98	0.99	0.69	0.99
6	LeFleur's Bluff	3	1.42	0.47	0.57	0.52
7	Crystal Lake	5	3.31	0.66	1.33	0.82
8	Georgetown	6	3.89	0.65	1.52	0.85
9	Atwood	5	3.46	0.69	1.41	0.88
10	Columbia	6	4.65	0.78	1.64	0.92
11	Bogalusa	5	1.68	0.34	0.85	0.53
12	Walkiah Bluff	4	2.35	0.59	1.01	0.73
13	Stennis	4	2.20	0.55	0.96	0.69
14	Bogue Chitto	6	4.14	0.69	1.57	0.88

The species richness calculations of, Simpsons Index (D), Simpsons Equitability (E_D), Shannon’s Diversity (H), and Shannon’s Equitability (J) for each site within the Pearl River Drainage.

Table 2.10 Pearl River Drainage Relative Abundances

Species	Overall	Lakes	Rivers
A. spinifera	0.11	0.15	0.09
C. p. dorsalis	0.00	0.00	0.00
C. serpentina	0.01	0.04	0.00
G. oculifera	0.02	0.00	0.03
G. pearlensis	0.07	0.00	0.10
M. temminckii	0.24	0.19	0.27
P. concinna	0.04	0.01	0.05
S. carinatus	0.11	0.07	0.13
S. odoratus	0.00	0.01	0.00
T. scripta	0.39	0.53	0.33

The relative abundance of all species within the Pearl River Drainage, overall, and then broken down by total trap nights on lake (lentic) sites, and riverine (lotic) sites.

Table 2.11 Big Black River Drainage Species Observed

Big Black River Drainage		Species Present								Total Diversity
ID	Site:	**A.m**	**A.s**	**G.oua**	**G.p.k**	**G.p.p**	**M.t**	**P.c**	**T.s**	
1	Goodman	1	1	0	1	0	1	0	1	5
2	Vaughan	1	1	0	1	1	1	1	1	7
3	Bentonia	1	1	1	1	1	1	0	1	7

The species observed at each site within the Big Black River drainage and at the Jourdan River site, as well as the total species for each site. (0 = species not observed, 1 = species observed).

Table 2.12 Big Black River Drainage Species Richness Measurements

ID #	Site	Species	D	E_D	H	J
1	Goodman	5	4.01	0.80	1.47	0.91
2	Vaughan	7	3.94	0.56	1.40	0.72
3	Bentonia	7	3.71	0.53	1.42	0.73

The species richness calculations of, Simpsons Index (D), Simpsons Equitability (ED), Shannon's Diversity (H), and Shannon's Equitability (J) for each site within the Pearl River Drainage.

Table 2.13 Big Black River Drainage Relative Abundances

Species	Relative Abundance
A. mutica	0.12
A. spinifera	0.23
G. ouachitensis	0.01
G. p. kohnii	0.09
G. p. pseudogeographica	0.04
M. temminckii	0.39
P. concinna	0.01
T. scripta	0.13

2.5 Figures

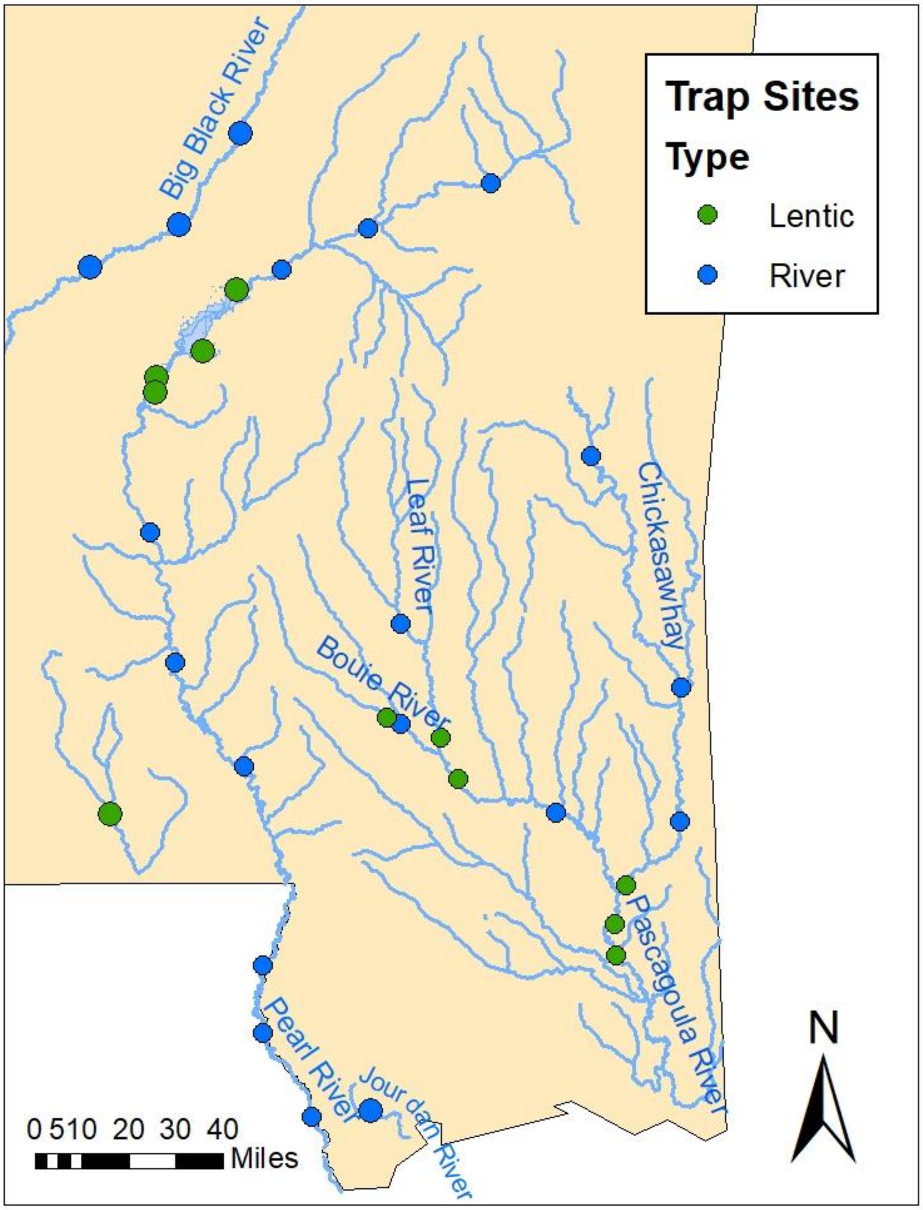

Figure 2.1 Survey Sites

A map depicting the four drainages that were sampled (Pascagoula River drainage, Peral River drainage, Big Black River, and the Jourdan River), as well as the general sampling locations. Each sampling location is colored green for lentic sites, and blue for river sites.

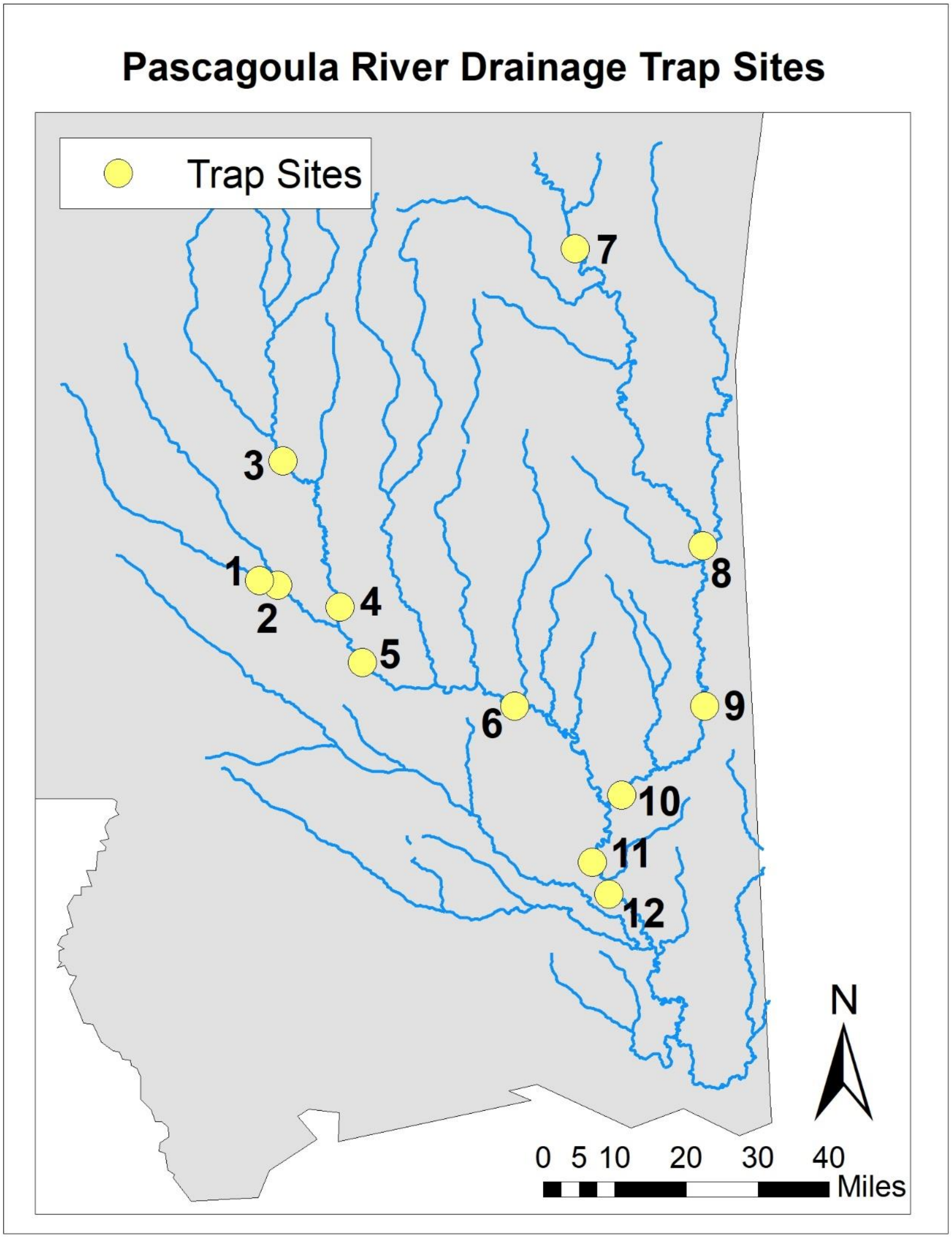

Figure 2.2 Pascagoula River Drainage Survey Sites

A map depicting the 12 locations surveyed within the Pascagoula River Drainage. The identification numbers for each site correspond to the identification numbers in Table 2.1, under the Pascagoula River Drainage section.

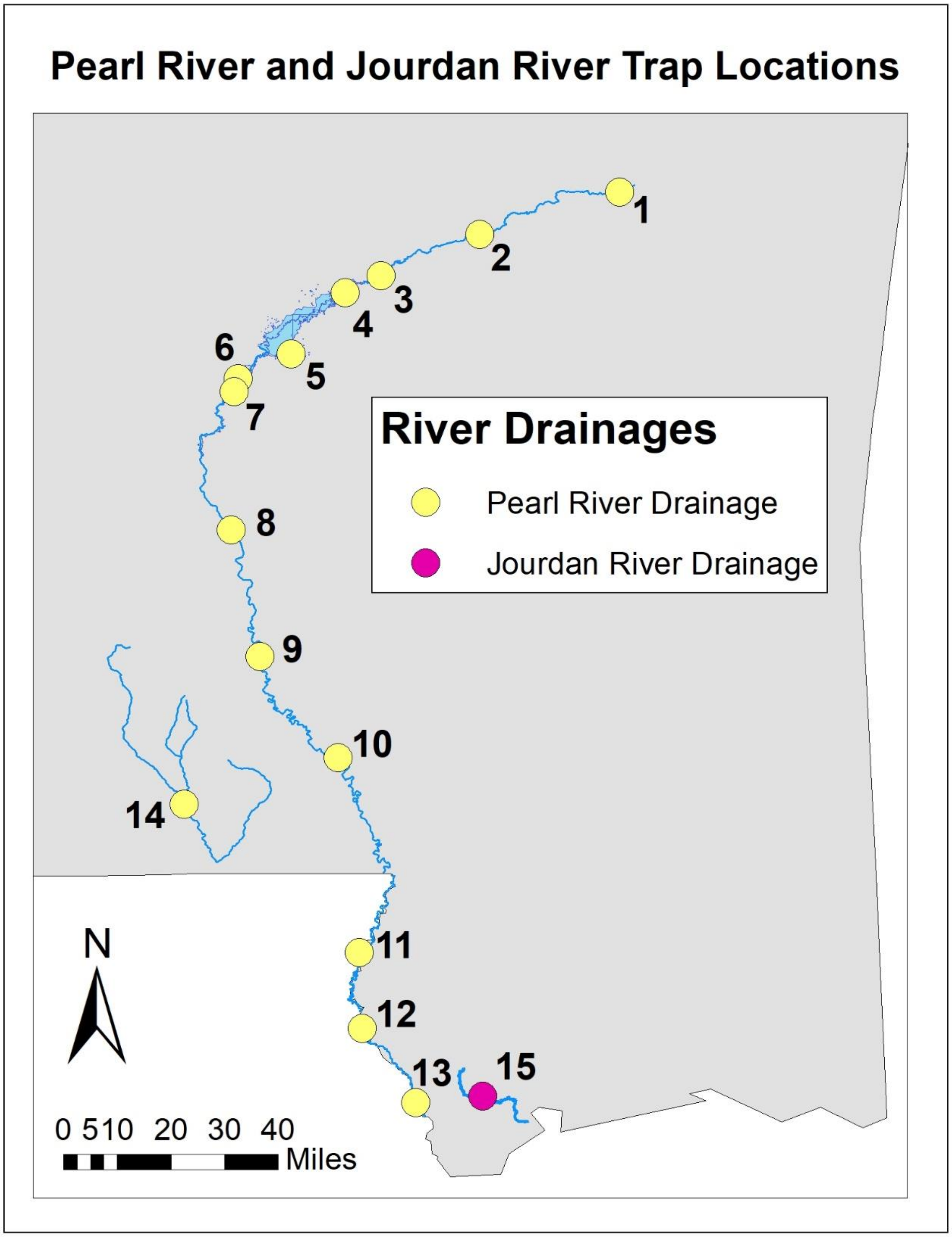

Figure 2.3 Pearl and Jourdan River Drainage Survey Sites

A map depicting the 14 locations surveyed within the Pearl River Drainage, and the single locality surveyed on the Jourdan River. The identification numbers for each site correspond to the identification numbers in Table 2.1, under the Pearl River Drainage and Jourdan River Section.

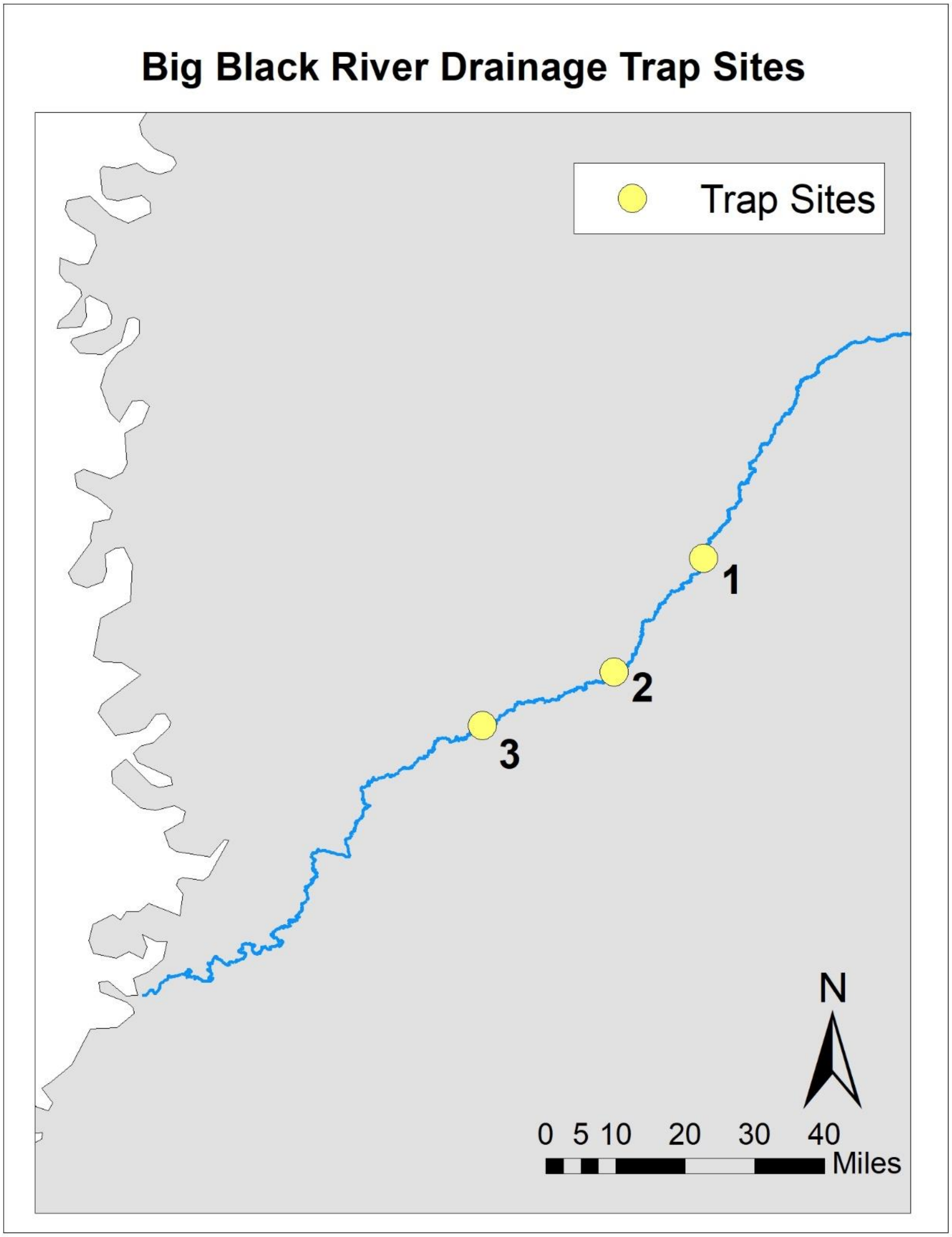

Figure 2.4 Big Black River Drainage Survey Sites

A map depicting the 3 locations surveyed within the Big Black River Drainage. The identification numbers for each site correspond to the identification numbers in Table 2.1, under the Big Black River Drainage Section.

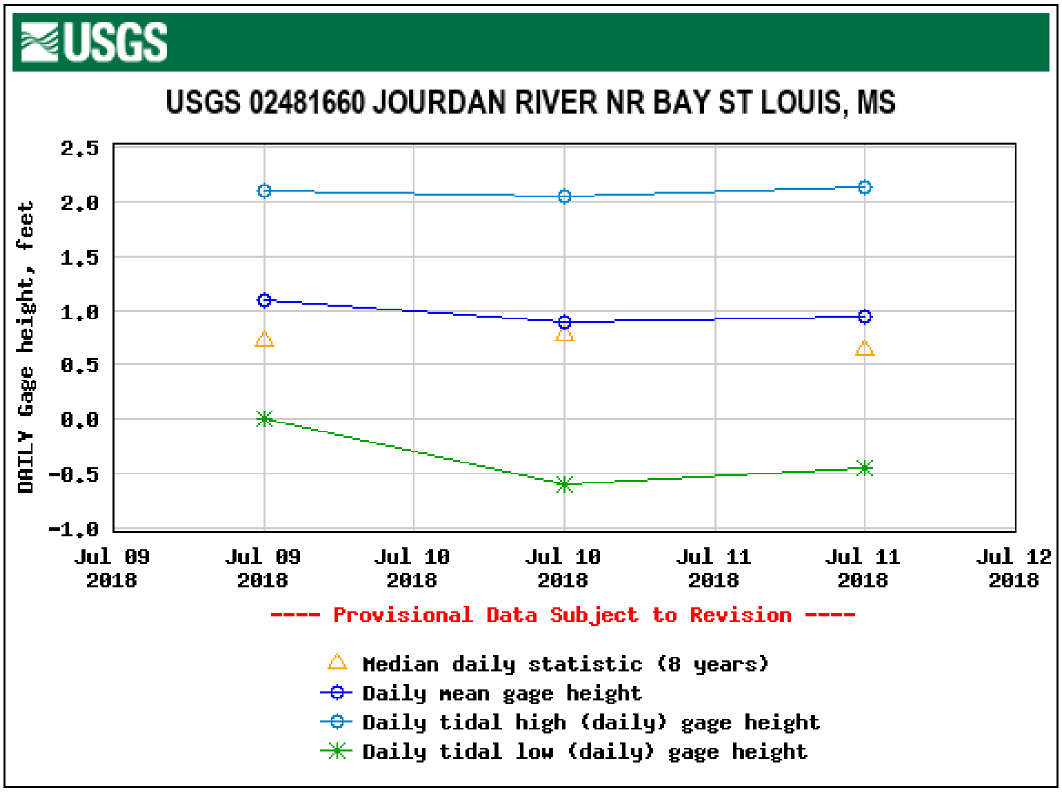

Figure 2.5 Jourdan River Tidal Statistics

The daily tidal statistics for the Jourdan River, which show the system fluctuated by around 2 feet as tides moved in and out.

2.6 Literature Citated

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CHAPTER III – HABITAT, LAND-USE, AND TURTLE COMMUNITIES OF SOUTH MISSISSIPPI

3.1 Introduction

As the impending rise of exportation for foreign trade looms on the horizon, and as habitat degradation and fragmentation increase, understanding what drives the species diversity and abundance of turtle communities on a large scale is becoming ever more important. This is especially true for turtles as a group, because their presence is paramount to the health of an ecosystem, as they can function as predators, prey, seed dispersers, habitat engineers, and nutrient cyclers (Lovich & Ennen, 2018). Previous studies have calculated the relative biomass of turtles within their ecosystems and on average turtles, especially those in freshwater environments, contribute a staggeringly high amount of biomass compared to other animal groups (Iverson, 1982; Congdon et al., 1986; DeGregoria et al., 2012). Biomass reflects the amount of available and stored energy in the plants and animals occupying an ecosystem (Lovich & Ennen, 2018), with higher biomass generally resulting in a greater overall impact. However, turtles have received very little attention for their critical role in their aquatic communities. Instead, fish and aquatic invertebrates have historically been the main groups studied to measure aquatic ecosystem health (Riedle, 2015). While the environmental importance of turtles is now being acknowledged, researchers are still behind the curve, as very few studies have tried to fully understand the direct effects of environmental factors on the aquatic turtle community makeup and relative abundance.

Numerous studies have measured the effect of habitat variation and surrounding land-use on aquatic or terrestrial ecosystem health using several organismal groups, including fish (Meador & Goldstein, 2003), macroinvertebrates (Sponseller et al., 2001), plants (Houlahan et al., 2006; Lougheed et al., 2001), and amphibian communities (Houlahan et al., 2003). The general consensus of these studies is that urbanization and/or agriculture degrades habitat and therefore leads to declines in native community and environmental health. However, very few studies have looked at land-use effects on aquatic turtle populations. Aquatic turtles have been observed as relatively hardy creatures, and can persevere in habitats where amphibians or fish would otherwise perish (Bridges & Semlitsch, 2001; Carey & Bryant, 1995; Packard, et al., 1997; Willmore & Storey, 1997). Thus, it is important to understand if changes in habitat use will have a noticeable effect on turtle communities like it does with other animal groups, or if turtles are able to persist where others cannot, perhaps because they are not as closely tied to the aquatic medium.

Likewise, in regions where extreme turtle diversity is present, like that of southern Mississippi, it is consequential that we have a thorough understanding of what can affect species diversity. Most importantly, we must understand whether turtles are as susceptible to changes in habitat and surrounding land-use as other aquatic animals, since this will allow for more informed management decisions aiming to preserve habitats which facilitate the high biodiversity found in Mississippi and throughout the southeastern United States. Our study aims to elucidate the environmental factors that drive the abundance and species make up of turtle communities by determining if environmentally similar trapping sites possess similar communities, and what environmental factors are important to each species. Similarly, we will determine if surrounding land cover has any effects on riverine turtle diversity or abundance. It is imperative that we begin to understand what can cause population growth or declines, as well as what external influences can lead to a shift in diversity, to better preserve the biodiversity of southern Mississippi.

3.2 Methods

3.2.1 Sampling

To capture turtles, hoop nets (90 cm diameter, 3-metal ring, and 120 cm diameter, 4-fiberglass ring) were partially submerged near suitable microhabitat (log jams, root masses, etc.) with the trap tied to a structure and/or a PVC pipe secured into the substrate and baited with fresh or frozen fish. A site constituted a stretch of river or lake, where approximately 23 nets were set, baited, and checked over a three to four-day period. We recorded all individuals of all species of aquatic turtles captured in each net at each site, performed morphometric measurements (carapace length, width, and height, plastron length, and mass), individually marked each turtle (Ernst, 1971), and released them all at their point of capture. Traps were checked daily, and basic habitat data was collected at each trap, including GPS coordinates, water current (no/ slow/ medium/ fast), canopy cover (densitometer), substrate % type (mud/ sand/ detritus/ vegetation/ gravel/ clay), water temperature (C°), stream width (m), distance to shore and distance to microhabitat (m), and type of microhabitats present in the direct vicinity of the trap (log jam, sandbar, root mass, etc.) to determine if specific variables coincided with different turtle capture rates.

3.2.2 Analyses

Our sampling focused heavily on Mississippi’s main southern drainages, and therefore we decided to focus the majority of our analyses on the sites from the Pascagoula River

Drainage and the Pearl River Drainage. To compare sites based on their habitat variables, two Principal Component Analyses (PCA) were performed, one for the Pascagoula River drainage and one for the Pearl River drainage. To determine the average eigenvalue score of each site, the eigenvalue scores from each set of traps reported by the Pascagoula PCA, and the Pearl PCA, were grouped by site and averaged. Two UPGMA cluster analyses were then performed on these new site PCA scores (a Pascagoula and Pearl analysis) to group the sites based on habitat similarities. Eigenvalues of the habitat variables were observed, and those with the greatest value were the variables deemed to be "driving factors". Then, to observe if turtle communities varied by grouping an ANOSIM analysis was performed on the clustered groups, with 5000 permutations (K = 3). A Non-metric Multidimensional Scaling analysis (NMDS) based on Bray Curtis dissimilarity was performed on the turtle communities of each clustered group to observe if communities differ based on habitat. All mentioned analyses were performed in the statistical program R. Finally, a One-factor Analysis of Variance (ANOVA), with the cluster site groupings as a fixed factor, was performed in program JMP on species richness and Shannon's diversity index to determine if measures of turtle diversity differ between habitat groupings. Before analyses were run, we checked the parametric test assumptions of normality using a Shapiro-Wilk test and equal variances using Bartlett's. If the ANOVA yielded a significant difference, a Tukey's HSD Post Hoc test was performed. If these assumptions were not met, a Kruskal-Wallace Rank Sum test was performed, and if significant differences arose, a Wilcoxon rank sum test was performed. We used three linear regressions to determine if drainage area, average site width, and the average monthly discharge corresponded to any trends in turtle diversity. A sequential

Bonferroni correction (Rice, 1989) was then performed to adjust statistical significance over multiple comparisons.

We used Canonical Correspondence Analysis (CCA), to test for significance and visual general patterns between species and habitat occupancy (Palmer, 1993; Riedle, 2015). The variance inflation factor (VIF) scores of all habitat variables were compared, and in the instance that two variables were deemed highly correlated, one was removed from the analyses. A one factor PERMANOVA with terms or axes as a fixed factor with 1000 permutations, was then performed to determine what habitat variables and gradients had a significant effect on turtle species. In our analyses, the habitat variables recorded at each trap location were compared to the species captured within the corresponding trap. We analyzed what habitat data is most strongly correlated with the presence to each species, what microhabitats are occupied by each species, and if microhabitat occupancy change depending on the sex of each species. These analyses were performed in the statistical program R.

Land use was determined using geographical information system (GIS) (ArcMap 10.6.1; ESRI, Redland, CA, U.S.A.) within a one-mile buffered radius of each trap’s GPS location. Digital land-cover data were obtained from the Mississippi Geospatial Clearinghouse. The land cover data from our 30 sites across Mississippi was originally grouped into 14 types of cover, including; developed – open space, developed – low intensity, developed – medium intensity, developed – high intensity, barren land, deciduous forest, evergreen forest, mixed forest, shrub, grassland, pasture, cultivated crop, woody wetlands, and emergent herbaceous wetlands. We further grouped these land cover types into one of three categories, including developed (developed – open space, developed – low intensity, developed – medium intensity, developed – high intensity, and barren land), forest (deciduous forest, evergreen forest, mixed forest, and woody wetland), and agriculture (grassland, pasture, and cultivated crop). To determine if land use influences turtle diversity and abundance, we compared the percent land use in these three categories of each site to the species richness and turtle abundances using linear regressions. The total linear length of roads present within these buffers was also quantified, and analyzed similarly to the other cover types. Finally, we used CCAs to test for significance and visualize associations between certain species and the surrounding land cover to determine if land use has any effect on community make up.

3.3 Results

3.3.1 Habitat Associations

3.3.1.1 Pascagoula River Drainage

Principal Component Analyses (PCA) results show the first and second axes of the Pascagoula sites explain 20.9% and 9.13% of site variability, respectively. The habitat variables river, width large (W – Large), no flow, medium flow (med), eddy, less than 5 meters to shore (BB), low canopy cover (HL), and river bend are strongly associated with axis 1 (Table 3.1). This axis represents the gradient from a lentic to lotic ecosystem. The variables under water structure low (UWS – L), gravel, basking structure presence (Bask – S), and agricultural surrounding land use (SL – Ag) are most strongly associated with axis 2 (Table 3.1), which likely represents the gradient of natural to more anthropogenically impacted sites. The eigenvalues of all traps were averaged across their sites and weighted based on percent variance explained, these site averages were then plotted based on the driving habitat factors. This resulted in sites clustering into three groups within the biplot (Fig. 3.2). A cluster analysis was performed on the first 6 axes (which together explained 53% of the variance) which yielded a similar outcome, with our six river sites grouping together, and our lentic sites splitting into two separate groups (Fig. 3.3).

When the groups and species relationships were plotted using an NMDS (Fig. 3.4 & 3.5) it is observable that Group 1, the riverine site grouping, is driven by a diverse community of numerous species. While Group 2 were lake sites inundated with Pond sliders, and Group 3 were lakes with Alligator Snapping turtles dominating the communities. The ANOSIM performed showed that there is a significant difference in the turtle community make-up between Group 1 and 2 ($R = 0.06$, $p = 0.003$), Group 1 and 3 ($R = 0.06$, $p = 0.005$), and Group 2 and 3($R = 0.06$, $p = 0.07$) (Fig. 3.5). However, these measures ® are low, and likely the significance represents differences in dispersion, not differences in community. Likewise, there were patterns in species richness with group 1 ($\bar{x} = 7.83$ species) possessing significantly more species ($\chi^2 = 9.014$, df = 2, $p = 0.011$) than groups 2 ($\bar{x} = 4$ species) and 3 ($\bar{x} = 4.3$ species). Likewise, group 1 ($\bar{x} = 1.63$) had a significantly greater score on the Shannon's Index ($F_{2, 9} = 123.5$, $p < 0.0001$) compared to both group 2 ($\bar{x} = 0.75$) and group 3 ($\bar{x} = 0.55$). However, there was no significant difference ($F_{2, 9} = 1.352$, $p = 0.307$) in the overall abundances of turtles.

3.3.1.2 Pearl River Drainage

Principal Component Analyses (PCA) results show the first and second axes of the Pearl sites explain 18.4% and 7.11% of site variability, respectively. The habitat variables; river, basking structure presence/ absence (BS – P/A), submerged vegetation, deadwood, and underwater structure (UWS – H/L) were most strongly associated with the first axis (Table 3.2), likely representing the gradient from a lentic to lotic ecosystem. Whereas, cypress knees, mud, slow flow (F – slow), high canopy cover (LL), medium canopy cover (ML), sandbar, medium flow (F – med), and a large width (W – large) are most strongly associated with the second axis (Table 3.2), likely representing a gradient of stream size. The eigenvalues of the first eight axes (which explain 53% of the variance) of all traps were averaged across their sites, these averages were then weighted by the percent of variance explained, and then these site averages plotted based on the driving habitat factors. This resulted in the sites clustering into three groups within the biplot (Fig. 3.6). The cluster analysis yielded a similar outcome, with lentic sites grouping together along the first and second axes, and river sites splitting into two groups along the second axis (Fig. 3.6 and 3.7).

When clustered groups are plotted based on turtle communities using a NMDS (Fig. 3.8 & 3.9) the communities of Group 1, which consists of lentic sites, are driven by the presence of *T. scripta* and *A. spinifera*. While Group 2, located central in the graph, possesses all species, but tends to have slightly greater numbers of *M. temminckii, G. pearlensis,* and *S. carinatus*, compared to Group 3 sites which possessed a large number of *M. temminckii* and *G. pearlensis*. There again were significant community differences between Group 1 and 2 (R = 0.05, p = 0.028), Group 1 and 3 (R = 0.05, p = 0.003), and Group 2 and 3 (R = 0.05, p = 0.021) (Fig. 4.9). However, there were no significant differences among the three groups in species richness ($\chi^2 = 3.07$, df = 3, p = 0.381), Shannon's Index ($F_{3, 10} = 1.42$, p = 0.295), or turtle abundances ($F_{3, 10} = 0.197$, p = 0.896).

Finally, the results of our three linear regressions show that the size, in this case determined by average stream width, of a site has no significant effect on turtle diversity

($F_{1,14} = 2.94$, $p = 0.109$) although the slope of the line is slightly negative ($m = -0.023$). However, both drainage area ($F_{1,14} = 11.28$, $p = 0.005$) and the average monthly discharge ($F_{1,14} = 9.08$, $p = 0.009$) have a significantly negative impact on species diversity.

3.3.2 Species Habitat Occupancy

3.3.2.1 Pascagoula River Drainage

The CCA analysis of the Pascagoula River drainage species microhabitat occupancy (Fig. 3.10) yielded 17% of variation explained and 83.1% of unconstrained variation, and a single significant axis (F = 17.967, p = 0.001), with an eigenvalue of 0.295. Of the 11 specified microhabitats (Table 3.3) only 5 were deemed significant, which included root mass (F = 4.768, p = 0.003), branches (F = 3.726, p = 0.026), emergent vegetation (F = 5.961, p = 0.001), sandbars (F = 4.855, p = 0.016), and emergent trees (F = 3.523, p = 0.022). Of the seven turtle species included in the analysis (Table 3.4), *A. mutica* was the most specialized as they were highly correlated with both axis 1 ($\lambda = 1.42$) and axis 2 ($\lambda = 2.17$), while *P. concinna* was the least specialized with low scores on both axis 1 ($\lambda = -0.07$) and axis 2 ($\lambda = -0.06$).

The analyses of the overall habitat yielded similar results (Fig. 3.11), with 28.3% of constrained and 71.7% of unconstrained variance explained, and the data overall having a significant effect of turtle species (F = 2.10, p = 0.001). Again, the first axis was significant (F = 27.51, p = 0.001), with an eigenvalue of 0.390. After correlated variables were removed a total of 26 habitat factors remained to analyze, of these only 3 were deemed significant (Table 3.5), these included; low underwater structure (F = 3.21, p = 0.035), underwater vegetation (F = 12.41, p = 0.001), and sand substrate (F = 3.11, p = 0.034). Similar to the microhabitat scores, *A. mutica* again showed the highest habitat specializations (Table 3.6) as the species was strongly correlated with both the first axis (λ = 1.00) and the second axis (λ = 2.02). However, unlike the microhabitat variables the second axis seems to have a much greater impact on *P. concinna* (λ = 1.26), while *A. spinifera* (λ axis 1 = 0.61, λ axis 2 = -0.23) and *S. carinatus* (λ axis 1 = 0.54, λ axis 2 = -0.22) show only slight correlations with the first axis, and the second axis seems to have very little impact.

3.3.2.2 Pearl River Drainage

The CCA analysis of the Pearl River drainage species microhabitat occupancy yielded 11.5% of constrained and 88.5% of unconstrained variance explained, with no significant axes. Of the 13 specified microhabitats (Table 3.7) only 2 were deemed significant, which included emergent vegetation (F = 3.25, p = 0.033) and the presence of basking structure (F = 2.90, p = 0.049). Of the six turtle species included in the analysis (Table 3.8) *P. concinna* was the most specialized with a high correlation to the second axis (λ = 2.71), while *M. temminckii* was the least specialized with low scores on both the first (λ = -0.27) and second axes (λ = 0.05).

Similarly, our analyses of the species overall habitat occupancy (Fig. 3.12) yielded 9.9% constrained and 90.1% unconstrained variance explained, and produced no significant axes (Table 3.9), with none of the 21 habitat factors deemed significant. However, species patterns were still present (Table 3.10), with *G. pearlensis* showing the greatest habitat specialization with high scores for both the first (λ = -2.69) and second axes (λ = -1.25), while *A. spinifera* showed the least amount of habitat specialization for both the first (λ = 0.300) and second axes (λ = -0.13).

3.3.2.3 Sex-based Habitat Occupancy

The CCA analysis of habitat occupancy based on sex (Fig. 3.13) focused on individuals captured within the Pascagoula River drainage, as Pearl River drainage sample sizes were too low for accurate analyses. Like previous analyses of the Pascagoula, this CCA yielded 34.7% of constrained and 65.3% of unconstrained variance explained, and a single significant axis (F = 16.21, p = 0.006), with an eigenvalue of 0.4921. Of the 44 total habitat factors (Table 3.11), 6 were deemed significant. These factors included a high amount of underwater structure (F = 3.01, p = 0.009), the presence of basking structure (F = 2.66, p = 0.034), underwater vegetation (F = 6.91, p = 0.001), medium water current (F = 2.29, p = 0.42), is the location designated as a river site (F = 2.24, p = 0.041), and the presence of a sand bar (F = 2.11, p = 0.045). Four others can be classified as being important, including mud substrate (F = 2.4, p = 0.058), canopy cover ranging from 25 to 75 LAI (F = 2.18, p = 0.064), slow current (F = 2.2, p = 0.063), and the area located at the bend of the river (F = 1.91, p = 0.096).

Of the 16 groups included in the analysis (Table 3.12), four in particular showed the greatest habitat specializations, including male *A. mutica,* male *G. gibbonsi,* juvenile *T. scripta,* and female *M. temminckii*. *Apalone mutica* and *G. gibbonsi* both showed high correlations with the first (λ *A.m.* = 1.47, λ *G. g.* = 1.51) and second (λ *A. m.* = -3.35, λ *G. g.* = -2.17) axes. Juvenile *T. scripta* likewise showed a high correlation with the first (λ = -2.22) and second (λ = -1.27) axes. However, juvenile *T. scripta* has an inverse relationship when compared to *A. mutica* and *G. gibbonsi*. Finally, female *M. temminckii* showed a strong positive correlation with the second axis (λ = 1.00) compared to the rest of the groups (Table 3.12).

3.3.3 Land Use Analyses

The CCA analysis of surrounding land cover effects on communities showed land use is overall a significant driver of turtle communities ($F_{3,30} = 3.65$, $p < 0.001$), as the first axis significantly effects communities ($F_{1,30} = 8.84$, $p < 0.001$). Of the 3 land use categories forest ($F_{1,30} = 4.9$, $p = 0.006$) and agriculture ($F_{1,30} = 4.95$, $p = 0.011$), seem to have the most significant impact on turtle communities. Of the seven turtle species included in the analysis (Table 3.13), *A. mutica* ($\lambda = 2.38$) and *S. carinatus* ($\lambda = -1.22$) were the most effected by the gradient of axis 1. These data are driven almost entirely by the sites within the Big Black drainage, as 68% of all *A. mutica* and zero *S. carinatus* were captured from these highly agricultural sites. Therefore, we decided to separate data by drainage and remove the Big Black from further analyses, as only three sites were trapped within that drainage.

The Pascagoula River drainage CCA analysis of land use and turtle communities showed surrounding land cover is not a significant driver of turtle communities within the drainage ($F_{3,14} = 0.917$, $p = 0.46$). However, our CCA analysis of surrounding land cover effects on the Pearl River communities showed land use does have a significant effect ($F_{3,14} = 2.1$, $p = 0.019$) on turtle communities within the drainage, as the first axis significantly effects certain species ($F_{1,14} = 5.10$, $p = 0.006$) (Table 3.14). Of the 3 land use categories, developed land ($F_{1,14} = 3.30$, $p = 0.015$) and forest ($F_{1,14} = 2.54$, $p = 0.026$) seem to have the most significant impact on turtle communities. Of the six turtle species included in the analysis (Table 4.14); *G. gibbonsi* ($\lambda = -1.79$) and *T. scripta* ($\lambda = 1.59$) were both highly correlated with axis one.

Overall, land use does not seem to be correlated with a change in turtle abundance or species richness. As percent of developed land had no significant effect on species richness ($F_{1,16} = 0.602$, $p = 0.4491$) or turtle abundance ($F_{1,16} = 2.32$, $p = 0.148$). Percent of forested land had no significant effect on species richness ($F_{1,16} = 0.583$, $p = 0.456$) or turtle abundance ($F_{1,16} = 0.2710$, $p = 0.6098$). And percent of agricultural land had no significant effect on species richness ($F_{1,16} = 0.575$, $p = 0.459$) or turtle abundance ($F_{1,16} = 0.0013$, $p = 0.972$). Finally, the length of road (m) within the site buffer showed no significant correlation with overall turtle abundance ($F_{1,28} = 0.452$, $p = 0.507$).

3.4 Discussion

The cluster analysis based on PCA habitat scores of the Pascagoula, clustered the 12 survey sites into three general habitat groupings (Fig. 3.2 and 3.3) with all river sites combining into a single group, and the remaining lake sites separating into two distinct groups. These lakes were likely separated based on anthropogenic impact, with Charles Deaton, Rhymes Lakes, and Pascagoula WMA sites all located in very natural unimpacted areas, whereas Murchinson Lake, Pierce Lake, and Wedgeworth are all located on private property and directly impacted by humans. And while there was no significant difference in the abundance of turtles at each site, the overall community make up did vary significantly between all three habitat groups (Fig. 3.4 and 3.5).

Similarly, the 14 Pearl sites congregate into three general groupings (Fig. 3.6 and 3.7). All lake sites clustered relatively cleanly into a single group, while river sites spread out along a vertical gradient which we believe is based on overall habitat. The second grouping consisted of Columbia, Bogue Chitto, Walkiah Bluff, Bogalusa, Carthage, and Atwood, which were all generally faster flowing, open, larger systems. While

Georgetown, Coal Bluff, Philadelphia, and Stennis were generally slower moving sites, surrounded by sloughs and backwaters, which formed the final grouping. When compared to the Pascagoula, the communities which drove Pearl River varied slightly, as the presence of *A. spinifera* was more geared towards lentic sights rather than lotic. However, river groupings from either drainage were generally driven by a diverse community of numerous species. And within Pearl River drainage in particular, while communities are significantly different from one another, a heavy overlap in community make-up does occur between the differing habitat groups (Fig. 3.9), which could be attributed to the lack of any easily predictable lentic versus lotic community make-up, like what was observed on the Pascagoula.

Community diversity at a particular place in time is thought to be the result of multiple driving processes, such as responses to abiotic factors (Connell, 1978), competitive interactions (Cody & Diamond, 1975), evolutionary specialization (Whittaker, 1972), species migration (MacArthur & Wilson 1967), overexploitation of prey resources by predators or disease (Morin, 1983), and species production (Prance 1982). Therefore, it seems that habitat make up alone is not an adequate predictor of turtle communities. However, our results show that while habitat cannot accurately predict overall community make-up, it can be a good predictor of species presence.

Similar to our site PCA, our Pascagoula species CCA had an axis that represented the gradient from lentic to lotic ecosystems (Fig. 3.10). This plot shows the majority of lotic species (*A. mutica, A. spinifera,* & *G. gibbonsi*) grouping together, while *T. scripta,* a species that prefers lentic habitat (Morreale & Gibbons, 1986), inhabits the opposite side of the axis. *M. temminckii,* a habitat generalist (Ernst & Lovich, 2009), can be found in the middle of axis 1, with a slightly greater association with lentic habitats. This pattern is likewise represented by microhabitat use (Fig. 3.11) with lotic species (*A. mutica*, *A. spinifera*, and *G. gibbonsi*) showing greater associations with microhabitats that almost exclusively occur in riverine environments, such as sandbars, branches, and large root masses, while *T. scripta* corresponds strongly with emergent vegetation and trees, microhabitats that are representative of lentic habitats like oxbow lakes, sloughs, and backwaters. *M. temminckii* and *S. carinatus*, both more or less habitat generalists (Ernst and Lovich, 2009), can be found in the center of the graph, correlated with roots or high cover areas, while *P. concinna* showed relatively little specific microhabitat association.

A similar, albeit less pronounced, pattern can be seen within the Pearl River drainage. While the first axis, again a gradient from lentic to lotic-based habitat was not significant, *T. scripta* still prefer lentic, and *G. pearlensis* and *S. carinatus* prefer lotic habitats (Fig. 3.12) which mirrors the patterns seen in the Pascagoula. *Macrochelys temminckii* again shows no specific habitat associations, as capture rates in both lentic and lotic habitats were similar. Surprisingly, *A. spinifera* showed a pattern similar to *M. temminckii*. While *A. spinifera* is generally thought to be a riverine species, they also inhabit ecotonal areas, small creeks, roadside and irrigation ditches, ponds, bayous, oxbows, large lakes, and impoundments (Ernst & Lovich, 2009).

We believe this pattern in *A. spinifera*, and the lack of significant habitat correlations within the Pearl, is due to the overall low abundance of turtles in the drainage, and possibly habitat homogenization. The Pearl River drainage has undergone a vast amount of localized urbanization within the Jackson area, pollution, removal of riparian buffer, and the construction of reservoirs (Ross Barnett Reservoir) and dams (Clark et al., 2018). Similarly, it has undergone localized channelization, dredging, de-snagging, and aggregate mining (Tipton et al., 2004). It has been shown that the degradation of natural habitat (Marchand & Litvaitis, 2004) and riverine alterations which aid in flood control (Usuda et al., 2012) have negative impacts on turtle populations. Overall, we captured significantly fewer turtles on the Pearl ($\bar{x}$ = 24.6 per site) compared to the Pascagoula ($\bar{x}$ = 53.8 per site).

The percentage of developed land surrounding the Pearl was, at some sites, staggering compared to the sites along the Pascagoula. For example, the highest development along Pearl sites occurred at Crystal Lake and LeFleur’s Bluff, with development covering 60% and 55% of the land, respectively, within the 1-mile buffer, compared to the Middle Leaf site with only 13% of land cover designated as developed, the highest on the Pascagoula. However, when averaged across all sites, the drainages show no significant difference in overall development, as much of the Pearl watershed outside the greater Jackson area, is relatively rural and covered in forest or agricultural fields. In fact, the significant effect of land use produced through the CCA was not the hypothesized negative impact of developed land on all species, but rather a strong positive correlation of *A. spinifera* with developed land, and *Graptemys* species with agriculture. The remaining species were grouped into the middle, with land use having minimal to no impact on their distribution or abundance. This pattern can likewise be seen in our Pascagoula land use CCA, which showed no real significance again with all species grouping towards the middle.

However, drainage area, specifically within the Pearl River, had an effect on turtle community diversity. As previously stated, the Pearl River has had numerous large-scale alterations, with the Ross Barnett Reservoir and Ross Barnett Dam arguably the most impactful. Man-made reservoirs or impoundments alone can have negative effects on native communities, as they tend to reduce diversity by homogenizing habitat (Vandewalle & Christainsen, 1996) and introducing exotic competitors, predators, and vectors for disease (Vannote et al., 1980). The purpose for creating impoundments, including that of Ross Barnett, is generally drinking water, recreation, and flow regulation (Cox, et al., 2011). Freshwater turtles depend on natural riverine hydrology and nesting habitat accessibility for sustainable population survival (Bodie, 2001). Previous research suggests that flow regulation can hinder turtle survival at multiple life stages, including high mortality in late stage embryos when water levels are artificially elevated during the summer (Tucker, et al., 1997), and juvenile mortality due to an artificial reduction in water levels during the winter (Bodie and Semlitsch, 2000). Likewise, there is a significant decrease in diversity as drainage area increases. In this case, drainage area represents the gradient of upstream sites to downstream, with sites above the reservoir, where flow is natural, having a greater diversity than those below the reservoir that have an unnatural flow regime. This pattern does not occur in the Pascagoula river sites, with diversity remaining relatively constant among all sites. However, a majority of the Pascagoula river sites are within the upper stretches of the drainage, which could affect these numbers, and survey sites from the lower stretches would be needed to better support this idea.

It seems habitat factors, and microhabitats readily show patterns and correlations with certain species. With species presence and abundance shifting significantly from lentic to lotic based habitats. However, habitat cannot accurately predict overall turtle community abundance or diversity. Similarly, surrounding land use seemed to have very little impact on turtle communities, diversity, or abundance. The Pearl seems to possess vastly fewer turtles, when compared to the Pascagoula. It is unknown whether populations have historically always been lower due to natural factors, or if anthropogenic effects have caused recent population declines. However, it is possible that unnatural flow regimes could be leading to a decrease in turtle abundance.

More research is necessary to understand the cause of the Pearl’s lower abundance of turtles. Likewise, the effects of surrounding land use are still very under studied in respect to its impact on turtle communities. More studies which include a larger number of sites from a single drainage and possess a greater range of land use percentages than those we obtained is needed to better understand the impact of surrounding land use on turtle communities.

3.5 Tables

Table 3.1 Pascagoula River Drainage Habitat Variable Eigenvalues

Habitat Variable	Axis 1	Axis 2
Underwater Structure High	-0.12	1.28
Underwater Structure Low	0.16	-1.24
Basking Structure	-0.63	0.52
UW Vegetation	1.05	-0.64
Sandbar	-1.30	-0.13
Mud	0.74	0.13
Sand	-0.99	-0.15
Veg	0.89	-0.61
Detritus	0.89	0.26
Gravel	-0.26	-0.10
Clay	-0.46	-0.10
> 5 m from Shore	-0.89	-0.05
> 5 m from Microhabitat	0.60	-0.17
High Light	-0.66	-0.18
Medium Light	0.052	-0.59
Low Light	0.62	0.85
No Flow	1.20	-0.01
Slow Flow	-0.48	0.17
Medium Flow	-0.39	0.14
Fast Flow	-0.15	0.14
Eddy	-0.56	-0.26
Flooded Forest	0.49	0.65
Tributary	0.01	-0.03
River	-1.46	-0.21
Oxbow	1.46	0.20
River Bend	-0.62	-0.04
Surround Land – Forest	0.01	0.57
Surrounding Land – Urban	0.20	-0.36
Surrounding Land – Agriculture	-0.08	-0.43
Width Small	0.38	-0.08
Width Medium	0.15	-0.51
Width Large	-0.77	0.35
Width Very Large	0.37	0.25

Habitat variables collected in the Pascagoula River drainage, and their relation to axis 1 or axis 2 of the PCA. The variables with the greatest absolute values are the most related to their corresponding axes.

Table 3.2 Pearl River Drainage Habitat Variable Eigenvalues

Habitat Variables	Axis 1	Axis 2
Mud	0.31	-0.84
Sand	-0.63	0.48
Detritus	1.01	0.20
Veg	0.93	0.56
Gravel	-0.27	0.30
Clay	-0.37	0.15
Logjam	-0.88	0.44
Deadwood	-1.18	0.02
Root mass	-0.83	0.17
Branches	-0.61	0.07
Emergent Vegetation	0.96	0.55
Sandbar	-0.78	0.67
Submerged Vegetation	1.24	0.54
Stump	0.054	0.17
Emergent Trees	0.57	-0.51
Cypress Knees	0.48	-0.87
Roots	-0.20	-0.58
Basking Structure Present	-1.26	0.07
Basking Structure Absent	1.26	-0.07
Underwater Structure High	-1.04	-0.27
Underwater Structure Low	1.04	0.25
High Light	0.57	-0.02
Medium Light	-0.10	-0.67
Low Light	-0.39	0.81
Width Small	0.32	-0.38
Width Medium	0.13	-0.45
Width Large	-0.44	0.35
Width Very Large	0.02	0.60
No Flow	1.01	0.14
Slow Flow	-0.17	-0.82
Medium Flow	-0.61	0.65
Fast Flow	-0.29	0.45
Eddy	-0.08	0.06
River	-1.42	-0.40
Lake	0	0

Table 3.2 (continued)

Creek	-0.03	-0.01
Backwater	0.92	0.31
Slough	0.26	-0.29
> 5 m from Shore	-0.68	-0.27
> 5 m from Microhabitat	0.01	0.26

Habitat variables collected in the Pearl River drainage, and their relation to axis 1 or axis 2 of the PCA. The variables with the greatest absolute values are the most related to their corresponding axes.

Table 3.3 Pascagoula River Drainage Microhabitat CCA Output

Microhabitat	Df	ChiSquare	F	Pr(>F)
Logjam	1	0.02	0.95	0.597
Deadwood	1	0.03	1.74	0.208
Root mass	1	0.08	4.77	0.003
Branches	1	0.06	3.73	0.025
Emergent Vegetation	1	0.10	5.96	0.003
Sandbar	1	0.08	4.86	0.014
Submerged Vegetation	1	0.02	1.09	0.504
Stump	1	0.01	0.85	0.526
Emergent Tree	1	0.06	3.52	0.019
Cypress Knees	1	0.02	1.31	0.39
Roots	1	0.02	1.23	0.251

The CCA output, and resulting significance of each microhabitat variable on turtle species within the Pascagoula drainage. Values will be slightly different than those reported due to multiple permutation outputs.

Table 3.4 Pascagoula River Drainage Microhabitat CCA Species Scores

Species	CCA1	CCA2
A. mutica	1.26	2.07
A. spinifera	0.61	-0.41
G. gibbonsi	1.28	-0.23
M. temminckii	-0.49	-1.40
P. concinna	0.23	0.95
S. carinatus	0.56	-0.37
T. scripta	-1.73	0.79

Species scores along the Pascagoula Microhabitat CCA axes. A higher absolute score along either, or both axes, shows a more specialized species, while low absolute scores show more of a generalist.

Table 3.5 Pascagoula River Drainage Habitat CCA Output

Habitat Variables	Df	ChiSquare	F	Pr(>F)
Underwater Structure Low	1	0.05	3.21	0.03
Basking Structure Present	1	0.04	2.33	0.14
Underwater Vegetation	1	0.20	12.40	0.001
Mud	1	0.03	1.90	0.20
Sand	1	0.05	3.11	0.04
Veg	1	0.01	0.48	0.90
Detritus	1	0.03	1.82	0.23
Gravel	1	0.04	2.66	0.06
Clay	1	0.02	1.15	0.47
<0.5 m from Shore	1	0.03	1.95	0.17
<0.5 m from Microhabitat	1	0.03	2.15	0.14
Low Light	1	0.03	1.60	0.29
Medium Light	1	0.03	1.71	0.25
Slow Flow	1	0.03	2.09	0.15
Med Flow	1	0.02	1.38	0.37
Fast Flow	1	0.01	0.37	0.94
Eddy	1	0.00	0.30	0.83
Flooded Forest	1	0.01	0.41	0.92
Tributary	1	0.01	0.50	0.86
Oxbow	1	0.01	0.53	0.79
River Bend	1	0.01	0.44	0.90
Surrounding Land Forest	1	0.02	1.03	0.52
Surrounding Lan Developed	1	0.01	0.85	0.59
Width Small	1	0.01	0.68	0.76
Width Medium	1	0.01	0.73	0.71
Width Large	1	0.01	0.67	0.79

The CCA output, and resulting significance of each habitat variable on turtle species within the Pascagoula drainage. Values will be slightly different than those reported due to multiple permutation outputs.

Table 3.6 Pascagoula River Drainage Habitat CCA Scores.

Species	CCA Axis 1	CCA Axis 2
A. mutica	1.00	2.02
A. spinifera	0.61	-0.23
G. gibbonsi	1.12	-0.4
M. temminckii	-0.18	-1.43
P. concinna	0.27	1.26
S. carinatus	0.54	-0.22
T. scripta	-1.91	0.44

Species scores along the Pascagoula Habitat CCA axes. A higher absolute score along either, or both axes, shows a more specialized species, while low absolute scores show more of a generalist.

Table 3.7 Pearl River Drainage Microhabitat CCA Output

Microhabitat	Df	Chi Square	F	Pr (>F)
Logjam	1	0.01	0.36	0.93
Deadwood	1	0.03	1.41	0.378
Root mass	1	0.01	0.3	0.952
Branches	1	0.004	0.22	0.977
Emergent Vegetation	1	0.07	3.25	0.035
Sandbar	1	0.05	2.21	0.139
Submergent Vegetation	1	0.02	0.8	0.674
Stump	1	0.02	0.75	0.718
Emergent Trees	1	0.03	1.18	0.482
Cypress Knees	1	0.05	2.36	0.102
Roots	1	0.01	0.37	0.911
Basking Structure Present	1	0.06	2.90	0.046

The CCA output, and resulting significance of each Pearl microhabitat variable on turtle species within the Pascagoula drainage. Values will be slightly different than those reported due to multiple permutation outputs.

Table 3.8 Pearl River Drainage Microhabitat CCA Species Scores

Species	CCA1	CCA2
A. spinifera	-1.55	-0.25
G. pearlensis	2.09	-0.06
M. temminckii	-0.27	0.06
P. concinna	0.28	2.71
S. carinatus	0.9	-1.05
T. scripta	-0.14	-0.42

Species scores along the Pearl Microhabitat CCA axes. A higher absolute score along either, or both axes, shows a more specialized species, while low absolute scores show more of a generalist.

Table 3.9 Pearl River Drainage Habitat CCA Output

Habitat Variables	Df	Chi-Square	F	Pr (>F)
Mud	1	0.01	0.46	0.872
Sand	1	0.03	1.24	0.447
Detritus	1	0.04	1.94	0.198
Veg	1	0.04	1.63	0.271
Gravel	1	0.004	0.18	0.988
Underwater Structure High	1	0.01	0.56	0.816
High Light	1	0.002	0.07	0.999
Medium Light	1	0.03	1.22	0.489
Width Small	1	0.04	1.55	0.303
Width Med	1	0.02	0.69	0.752
Width Large	1	0.006	0.28	0.957
No Flow	1	0.005	0.20	0.978
Slow Flow	1	0.02	0.88	0.657
Medium Flow	1	0.006	0.27	0.972
Fast Flow	1	0.07	2.96	0.056
River	1	0.06	2.66	0.073
Backwater	1	0.02	0.78	0.681

The CCA output, and resulting significance of each habitat variable on turtle species within the Pearl drainage. Values will be slightly different than reported due to multiple permutation outputs. No values within this analysis were deemed significant.

Table 3.10 Pearl River Drainage Habitat CCA Species Scores

Species	CCA1	CCA2
A. spinifera	0.30	-0.14
G. pearlensis	-2.69	-1.25
M. temminckii	0.51	0.25
P. concinna	-0.62	0.76
S. carinatus	-0.27	1.7
T. scripta	0.88	-1.23

Species scores along the Pearl Habitat CCA axes. A higher absolute score along either, or both axes, shows a more specialized species, while low absolute scores show more of a generalist.

Table 3.11 Species Sex-based CCA Output

Habitat Variables	Df	Chi Square	F	Pr (>F)
Underwater Structure High	1	0.11	3.01	0.007
Underwater Structure Low	1	0.07	1.84	0.157
Basking Structure Present	1	0.1	2.66	0.016
Underwater Vegetation	1	0.26	6.91	0.001
Sandbar	1	0.07	1.95	0.144
Mud	1	0.09	2.4	0.039
Sand	1	0.06	1.63	0.285
Veg	1	0.03	0.80	0.858
Detritus	1	0.07	1.81	0.202
Gravel	1	0.06	1.66	0.226
Clay	1	0.02	0.56	0.974
> 5 m from Shore	1	0.05	1.41	0.42
> 5 m from Microhabitat	1	0.06	1.61	0.293
Low Light	1	0.06	1.49	0.346
Medium Light	1	0.08	2.18	0.082
No Flow	1	0.05	1.27	0.504
Slow Flow	1	0.08	2.2	0.058
Medium Flow	1	0.09	2.29	0.049
Fast Flow	1	0.02	0.64	0.786
Eddy	1	0.01	0.32	0.919
Flooded Forest	1	0.02	0.60	0.938
Tributary	1	0.04	1.06	0.451
River	1	0.08	2.243	0.054
Oxbow	1	0.03	0.79	0.649
River Bend	1	0.07	1.91	0.122
Surrounding Land use - Forest	1	0.02	0.64	0.923
Surrounding Land use - Urban	1	0.05	1.22	0.401
Width Small	1	0.02	0.67	0.928
Width Medium	1	0.05	1.21	0.522
Width Large	1	0.05	1.41	0.35
Logjam	1	0.05	1.20	0.512
Deadwood	1	0.04	1.13	0.552
Root mass	1	0.06	1.74	0.162
Branches	1	0.04	1.10	0.542
Emergent Vegetation	1	0.02	0.49	0.979
Sandbar	1	0.08	2.11	0.045
Submergent vegetation	1	0.03	0.76	0.856
Stump	1	0.03	0.94	0.538
Emergent Trees	1	0.01	0.39	0.99
Cypress Knees	1	0.03	0.79	0.844
Roots	1	0.04	1.16	0.337

Table 3.12 Pascagoula River Drainage Sex-based Species Scores

Species by Sex	CCA1	CCA2
A. mutica - Male	1.47	-3.35
A. mutica – Female	0.90	1.43
A. spinifera – Male	0.64	0.32
A. spinifera – Female	0.68	0.03
G. gibbonsi – Male	1.51	-2.17
G. gibbonsi – Female	1.16	1.3
M. temminckii – Male	-0.46	0.46
M. temminckii – Female	-0.63	1.00
M. temminckii – Juvenile	0.15	0.72
P. concinna – Male	0.20	0.38
P. concinna – Female	0.29	-0.95
S. carinatus – Male	0.74	0.15
S. carinatus – Female	0.95	0.53
T. scripta – Male	-1.20	-0.24
T. scripta – Female	-1.48	-0.37
T. scripta - Juvenile	-2.22	-1.21

Species scores, separated by sex, along the Pascagoula habitat CCA axes. A higher absolute score along either, or both axes, shows a more specialized species, while low absolute scores show more of a generalist.

Table 3.13 Land Use CCA Analysis Species Scores

Species	CCA1	CCA2
A. mutica	2.38	-0.01
A. spinifera	0.1	0.84
G. gibbonsi	0.99	-0.82
M. temminckii	-0.06	-0.10
P. concinna	-0.67	-1.19
S. carinatus	-1.21	-0.79
T. scripta	-0.59	1.88

Species scores along land use CCA axes which included all surveyed sites from our 2017 and 2018 surveying seasons. A higher absolute score along either, or both axes, shows a species is more likely to be found in a particular area, while low absolute scores show a species that can found equally abundant in any site.

Table 3.14 Pearl River Drainage Land Use CCA Analysis Species Scores

Species	CCA1	CCA2
A. spinifera	0.78	1.43
G. gibbonsi	-1.79	0.90
M. temminckii	-0.05	-1.03
P. concinna	-0.49	-1.34
S. carinatus	-0.20	0.37
T. scripta	1.59	-0.26

Species scores along land use CCA axes of the Pearl drainage. A higher absolute score along either, or both axes, shows a species is more likely to be found in an area surrounded by a particular land cover, while low absolute scores show a species that can found equally abundant in any site.

3.6 Figures

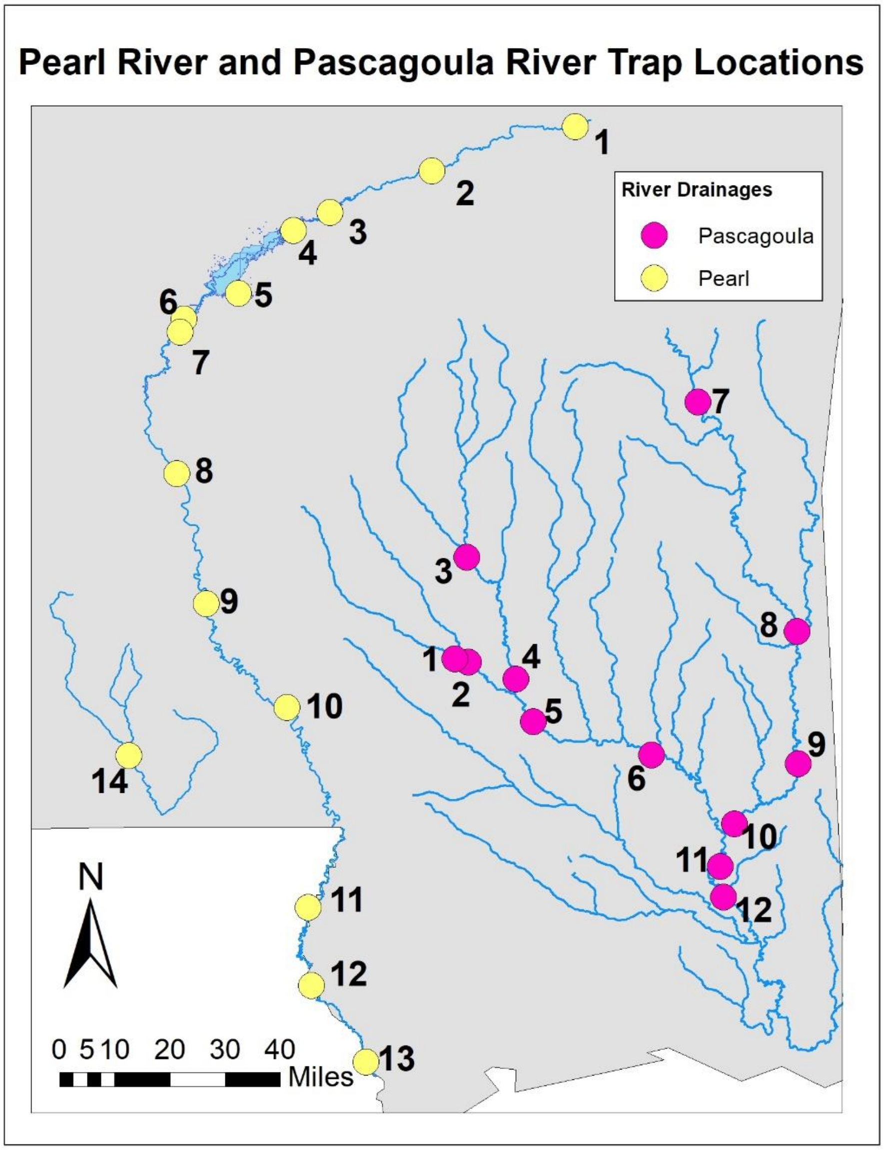

Figure 3.1 Sites Surveyed within the Pascagoula and Pearl River Draianges

We trapped 12 sites along the Pascagoula, and 14 sites along the Pearl for a total of 26 sites.

Pascagoula Sites: 1) Murchinson Lake, 2) Upper Bouie, 3) Upper Leaf, 4) Pierce Lake, 5) Wedgeworth, 6) Middle Leaf, 7) Upper Chickasawhay, 8) Middle Chickasawhay, 9) Lower Chickasawhay, 10) Charles Deaton, 11) Pascagoula WMA, and 12) Rhymes Lakes.

Pearl Sites: 1) Philadelphia, 2) Carthage, 3) Coal Bluff, 4) Ross Barnett North, 5) Ross Barnett South, 6) LeFleur’s Bluff, 7) Crystal Lake, 8) Georgetown, 9) Atwood, 10) Columbia, 11) Bogalusa, 12) Walkiah Bluff, 13) Stennis, and 14) Bogue Chitto.

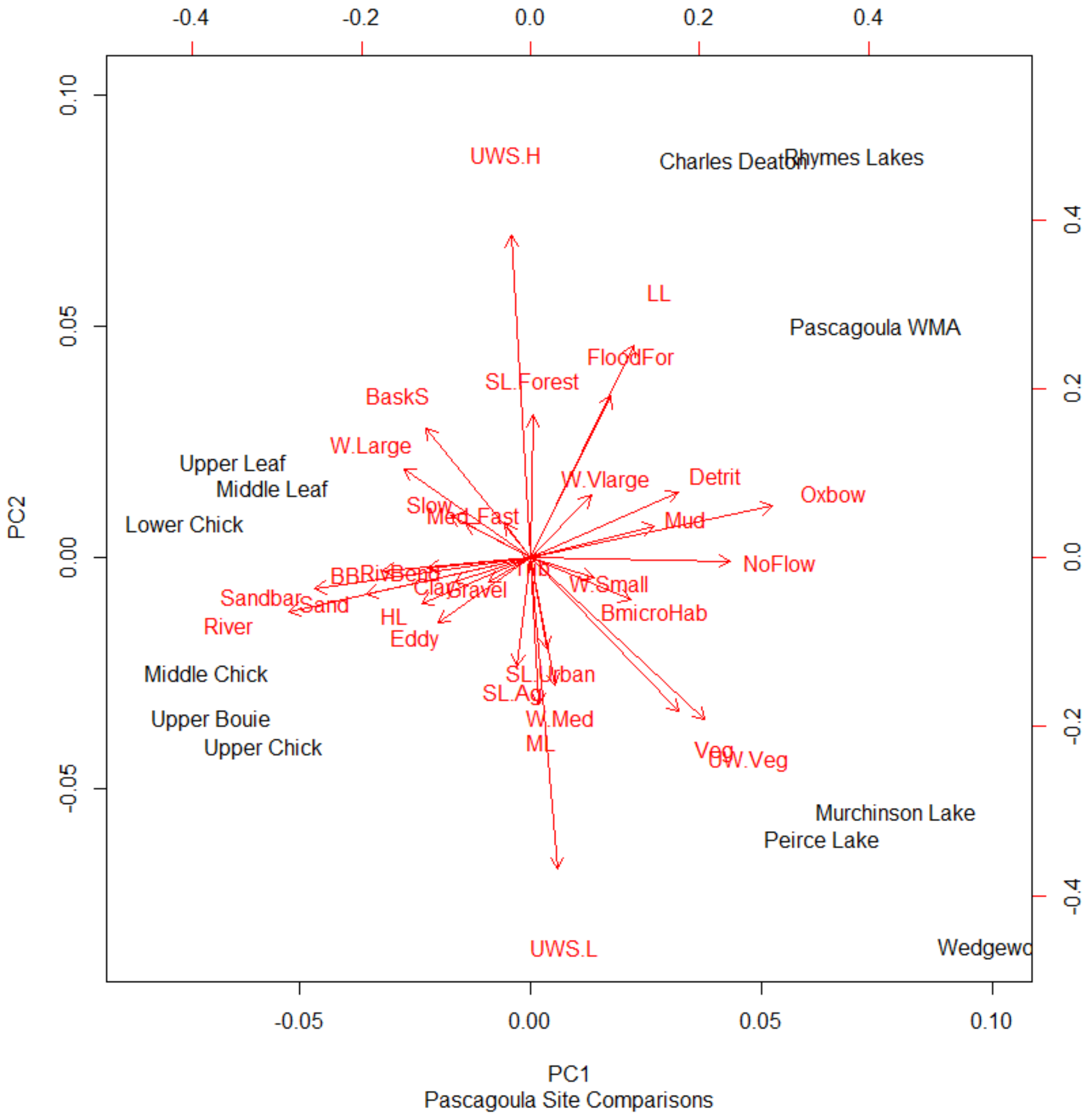

Figure 3.2 Pascagoula River Drainage PCA Biplot

PCA biplot of Pascagoula Sites, with the six river sites clustering into a single group on the left, and the 6 lake sites clustering into two groups on the right.

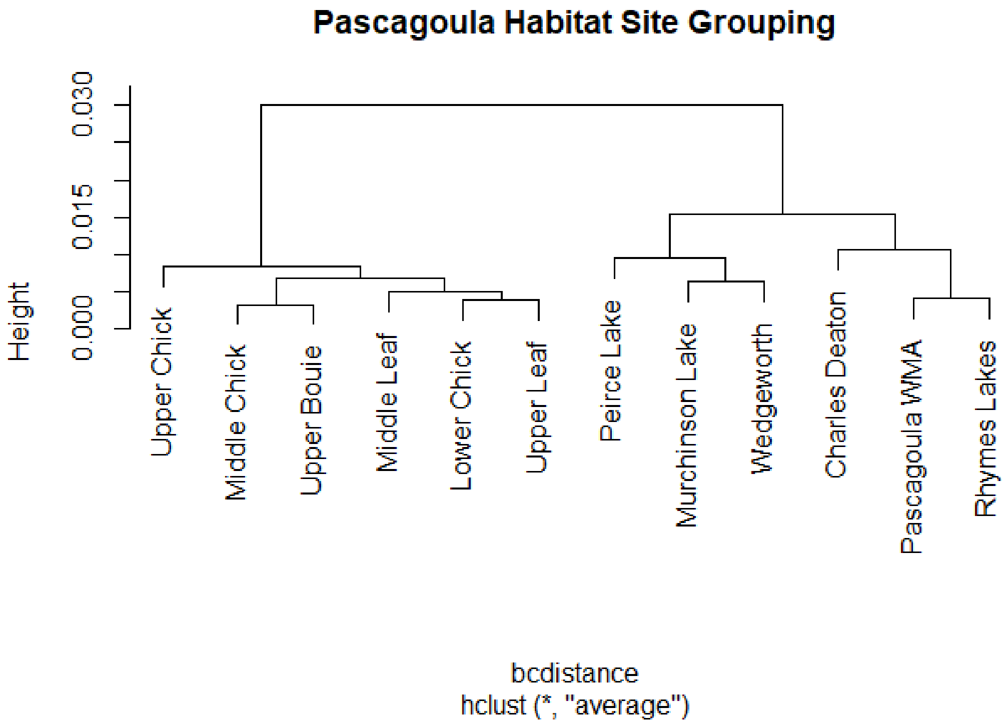

Figure 3.3 Pascagoula River Drainage Cluster Analysis

Cluster analysis, which grouped our Pascagoula sites together based on six axes of habitat similarity. This cluster plot further supports our 3 site groupings in our PCA biplot (Figure 4.2).

Group 1: Upper Chick, Middle Chick, Upper Bouie, Middle Leaf, Lower Chick, & Upper Leaf

Group 2: Peirce Lake, Murchinson Lake, & Wedgeworth

Group 3: Charles Deaton, Pascagoula WMA, Rhymes Lakes

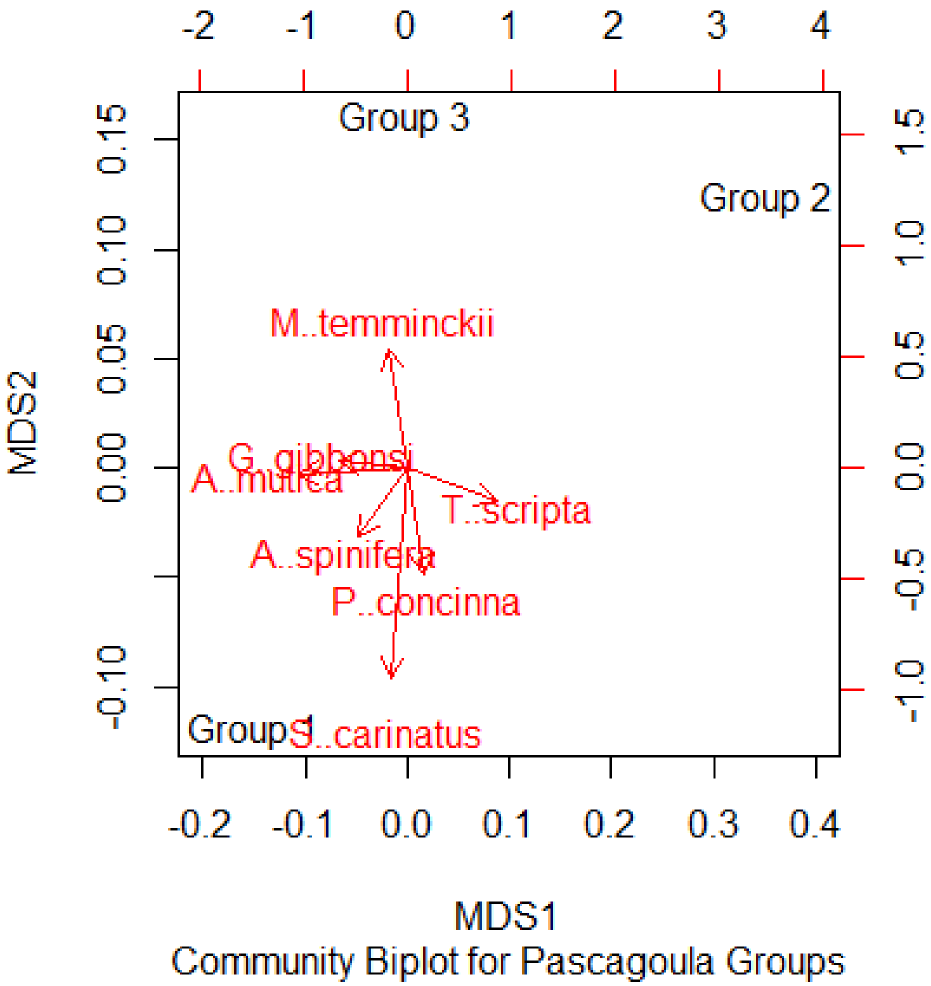

Figure 3.4 Pascagoula River Drainage Turtle Communities NMDS

NMDS biplot of Pascagoula turtle communities across our 3 cluster groupings of Pascagoula sites. Group 1 has a diverse community that is characterized by the presence of numerous, generally lotic species. While the community of Group 2 is dominated by T. scripta, and the communities of Group 3 show a large number of M. temminckii.

3.7 Literature cited

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CHAPTER IV – POPULATION GENETICS OF THE SPINY SOFTSHELL TURTLE (*APALONE SPINIFERA*) IN SOUTHERN MISSISSIPPI, COMPARING THE PASCAGOULA RIVER DRAINAGE TO THE PEARL RIVER DRAINAGE

4.1 Introduction

The family *Trionychidae*, or soft-shelled turtles, is an extremely unique group. This is mainly due to the striking reduction of the armored bony shell, which is stereotypical of almost all other turtles. This group can be specifically characterized by a flexible bridge region, the loss of peripherals, and both a carapace and plastron no longer covered by keratinous scutes, but with a leathery skin (Scheyer et al., 2007). While the origin of the turtle shell is thought to have arisen from the greater strength and stability enlarged ribs provided for burrowing (Joyce et al., 2009), it is believed that the secondary loss or reduction present in softshells allowed for overall greater mobility and thus greater speed in an aquatic environment (Scheyer et al., 2007). Presently, there are 13 genera and 31 living species of softshells (Fritz and Havas, 2007), and of those, three species (Florida softshell [*Apalone ferox*], Smooth softshell [*A. mutica*], and Spiny softshell [*A. spinifera*]) can be found within the continental United States.

Of these three species, *A. spinifera* has by far the largest range, naturally spanning from Canada south into Mexico, and throughout 37 eastern and mid-western states (Iverson and Mittermeier, 2010). *Apalone spinifera* can be distinguished from other *Apalone* by the conical spiny projections present along the anterior edge of the carapace, the sandpaper texture that is sometimes present in the dorsal integument, and the presence of a nasal septum (Ernst and Lovich, 2009). Currently, *A. spinifera* has been split into six subspecies, some of which have expansive ranges such as the Northern Spiny softshell

(*A. s. spinifera)* which can be found in 30 U.S. states and Canada, while others can be restricted to a single river drainage (Texas Spiny softshell (*A. s. emoryi)),* or even a single constrained locality (Black Spiny softshell (*A. s. atra*)). However, the exact range and validity of each subspecies has been debated in the literature (McGaugh et al., 2007; Weisrock & Janzen, 1999; McGaugh, 1999; Rhodin et al., 2017).

Three subspecies, all broad ranging, are thought to inhabit the state of Mississippi, including the Gulf Coast Spiny softshell *(A. s. aspera)* present in the northeastern and southern river drainages, such as the Tombigbee, Pascagoula, and Pearl Rivers, the Eastern Spiny softshell (*A. s. spinifera*) found in northwestern systems, such as the Yazoo, and the Pallid Spiny softshell (*A. s. pallida)* which inhabits the Mississippi River. However, most of these ranges have been delineated using physical characters of specimens alone, and the presence of a large intergrade area at the confluence of the Yazoo and Big Black rivers with the Mississippi River (Iverson and Mittermeier, 2010) creates ambiguity in terms of distributional patterns. It is important that the subspecies present within Mississippi are identified, and their ranges fully understood, as patchy or incomplete records can hinder appropriate conservation decisions (Selman & Qualls, 2009).

It is concerning that no analyses of genetic structure have been conducted on *A. spinifera* within the state of Mississippi, as many of Mississippi's rivers show high rates of endemism, specifically within the *Graptemys* genus. This is particularly true within the neighboring Pascagoula and Pearl River drainages where recent genetic work has split two cryptic members of the genus *Graptemys*, *G. pearlensis* and *G. gibbonsi*, that were once believed to be a single species (Ennen et al., 2010). Different clades that inhabit the same region should generally show congruent patterns of genetic structure over time and space (Wiley & Mayden, 1985). With this in mind, we have reason to believe genetic differentiation among rivers, like that seen in *Graptemys*, may be present in other riverine species as well, including the Spiny softshell. Furthermore, intra-drainage population genetic structure might also be present such as that exhibited by *G. flavimaculata* (Selman et al. 2013) and *G. oculifera* (Gaillard et al. 2016). Genetic differentiation within a drainage wouldn't be unexpected in *A. spinifera* as a study of *A. s. emoryi* in the Rio Grande River detected a pattern of isolation by distance (Mali, et al., 2015).

Our study focused on the state's large southern drainages, the Pascagoula and Pearl Rivers. The Pascagoula River drainage is the largest un-altered river system in the lower 48 United States (Dynesius and Nilsson, 1994). This is in stark contrast to the Pearl River, which has experienced extensive localized urbanization within the Jackson area, pollution, removal of riparian buffer, and the construction of reservoirs (Ross Barnett Reservoir) and dams (Clark et al., 2018). Similarly, it has undergone localized channelization, dredging, de-snagging, and aggregate mining (Tipton et al., 2004). The drainages likewise vary considerably in hydrogeography, as the Pascagoula River is highly dendritic in nature in contrast to the linear Pearl River drainage. We tested for inter-drainage genetic structure to see if, like the *Graptemys* species, *A. spinifera* may show inter-drainage genetic differentiation. Similarly, we tested for intra-drainage structure which, if present, could allow us to make inferences on the possible impacts of anthropogenic alterations of the river on genetic structure, as well as the effects a dendritic versus non-dendritic system could impose.

4.2 Methods

Turtle communities were sampled from 12 sites within the Pascagoula River drainage, and 14 sites within the Pearl River drainage. A total of 140 *Apalone spinifera* were captured using baited hoop nets (90 cm diameter, 3-metal ring, and 120 cm diameter, 4-fiberglass ring) partially submerged near suitable microhabitat (log jams, root masses, etc.). Traps were baited with frozen or fresh fish and checked within 24 hours of setting. All captured turtles were identified to species, sex was determined, and all were uniquely marked. *Apalone* were marked using unique tattoo IDs using a battery-operated tattooing gun (Inkinator cordless;Weber et al, 2011). Tissue was acquired from the outer carapace using a 5 mm biopsy punch, stored in 100% ethanol, and then stored in a freezer. Turtles were released at the point of capture.

Genomic DNA was extracted from tissue samples using a DNeasy Tissue Kit (QIAGEN Inc., Valencia, CA). Each individual was genotyped for seven microsatellite loci (*As12*, *As13*, *As15*, *AsB07*, *AsB08*, *AsB12* and *AsB14*) described by Davy et al. 2012. Each locus was amplified via the polymerase chain reaction (PCR) using a reaction mixture of 1.5–2.0 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.01% gelatin, 200 μM dNTPs, 0.1 μM of M13-labelled primer (LI-COR), 0.3 μM of M13-tailed forward primer (Boutin-Ganache et al. 2001), 0.3 μM of reverse primer, 0.1875 units of *Taq* polymerase (New England Biolabs), 20–100 ng of template DNA and water to a final 12.5 μL volume. PCR products were visualized on a LI-COR 4300 DNA Analyzer with a 50-350 bp size standard (LI-COR). GeneProfiler 4.05 (Scanalytics Inc.) was used to score allele sizes.

For purposes of genetic analyses, Pascagoula River individuals were pooled into seven different groups (Fig. 4.1): Bouie River (n = 22), Upper Leaf (n = 18), Middle Leaf (n = 3), Upper Chickasawhay (n = 19), Middle Chickasawhay (n = 22), Lower Chickasawhay (n = 7), and Pascagoula Proper (n = 2). Similarly, Pearl River individuals were pooled into five different groups (Fig. 4.2): Upper Pearl (n = 6), Reservoir area (n = 19), Middle Pearl (n= 9), and Lower Pearl (n = 9), and the Bogue Chitto (n = 1). Loci were screened for linkage disequilibrium and Hardy-Weinberg equilibrium within each site by GenePop on the web (Raymond and Rousset 1995), using a sequential Bonferroni correction (Rice 1989) to adjust statistical significance over multiple comparisons for a total alpha of 0.05. Basic summary statistics (number of alleles (N_A), observed heterozygosity - H_o and expected heterozygosity - H_e) were calculated using GenAlEx 6.501 (Peakall and Smouse, 2006; 2012). A Mantel's test as performed by GenAlEx was used to determine if there isolation by distance as evidenced by a positive relationship between geographic and genetic distance. Geographic distances were calculated as river distance (rkm) between sites while genetic distance was represented by F_{ST}. Pascagoula River drainage geographic distances were calculated using the line measure function in Google Earth, to obtain river distances. Pearl River drainage geographic distances were calculated with the GenAlEx Excel program, using the geographic distance function, based on GPS points taken at each site. Geographic distances were calculated differently for the separate drainages due to the fact the Pascagoula River drainage is a highly dendritic system, whereas the Pearl River drainage is extremely linear. GenAlEx calculates geographic distance based on the straight-line distance between GPS points. This gives an acceptably accurate measurement of river distance in the Pearl River drainage, but could not be accurately used for the Pascagoula River drainage. Both the Pascagoula Proper and Middle Leaf groupings were excluded from the Pascagoula River drainage Mantel analyses, and the Bogue Chitto were excluded from the Pearl drainage Mantel analyses as sample size was deemed too low.

The program STRUCTURE 2.3.4 (Pritchard et al. 2000) was used to determine if population genetic structure existed between and within the Pearl and the Pascagoula Rivers. STRUCTURE uses a Bayesian approach to partition individuals into some number of genetically discrete populations that are in Hardy-Weinberg and linkage equilibrium. The number of populations (K) from 1-6, were tested with 20 replicates each using a model of no admixture, assuming correlated allele frequencies between groups and with site location used as a prior (Hubisz et al. 2009) to observe if population structure was present between the Pascagoula and Pearl River drainages. A burn-in of 150,000 generations was followed by a subsequent 100,000 generations. The best value of K was determined by comparing the mean log likelihood scores for each value of K and by examining the ΔK values (Evanno et al. 2005) calculated by the program Structure Harvester v 6.92 (Earl and von Holdt, 2012). To search for intra-drainage structure individuals from just the Pascagoula or Pearl River sites were also analyzed with STRUCTURE using the same parameters.

4.3 Results

A total of 137 *A. spinifera* were used in our genetic analysis. Of these individuals, 94 were captured from May to September 2017 from the Pascagoula River drainage (Fig. 4.1), and 43 were captured from May to September 2018 from the Pearl River drainage

(Fig. 4.2). No loci deviated from Hardy-Weinberg equilibrium or demonstrated linkage disequilibrium after a sequential Bonferroni correction.

Measures of genetic diversity were fairly consistent across drainages (Table 4.1), with the mean number of alleles per locus for the Pascagoula River drainage ($N_a = 6.28$, SE = 1.13) varying only slightly from the Pearl River drainage ($N_a = 5.71$, SE = 0.78). Likewise, mean observed and expected heterozygosity values of the Pascagoula River drainage ($H_o = 0.584$, $H_e = 0.572$) were very similar to those of the Pearl River ($H_o = 0.574$, $H_e = 0.582$). The mean number of alleles per locus per group from within the Pearl River drainage (Table 4.2) ranged from 3.286 - 4.571. While the mean observed and expected heterozygosity values ranged from 0.401 - 696 and 0.447 - 0.599, respectively. Pascagoula River populations (Table 4.4) showed similar patterns, as the mean number of alleles per locus per group ranged from 2.714 - 4.714. And the mean observed and expected heterozygosity values ranged from 0.551 - 0.714 and 0.411 - 0.593, respectively.

The STRUCTURE analysis of the Pascagoula and Pearl River drainages found evidence of genetic differentiation between drainages, with K = 2 having the highest mean likelihood score (Figure 4.3 & 4.4; mean LnP(K) = -2220.93; SD = 1.01). The STRUCTURE analysis found no additional population genetic structure within the Pearl River as K = 1 had the highest mean likelihood score (Figure 4.6; mean LnP(K) = -715.47; SD = 0.889). However, there was some degree of genetic differentiation among sites with F_{ST} values ranging from 0.027 (Upper Pearl-Reservoir area) to 0.080 (Upper Pearl-Lower Pearl) (Table 4.3). Overall, genetic differentiation among sites reflected the geographic distance between them and the Mantel test revealed a pattern of isolation by distance through a positive correlation between geographic and genetic distance (Figure 4.5; r = 0.906, P = 0.044). Similarly, the Pascagoula River drainage STRUCTURE analysis found no evidence of multiple genetically distinct populations with K = 1 having the highest mean likelihood score (Figure 4.8; mean LnP(K) = -1490.93; SD = 0.684). Genetic differentiation (Table 4.5) among sites (F_{ST}) ranged from 0.007 (Upper Leaf-Upper Chick) to 0.119 (Middle Leaf-Pascagoula Proper). Generally, genetic differentiation among sites did not reflect the geographic distance between them, as the Mantel test showed no significant correlation between populations geographic and genetic difference (Figure 4.7; r = 0.165, P = 0.11).

4.4 Discussion

The inter-drainage structure analysis of *A. spinifera* from the Pascagoula and Pearl River drainages found some degree of genetic differences among populations with an overall K of 2 (Fig. 4.3 & 4.4). This finding was expected as the allopatry of these two populations mirrors that of the Pascagoula Map turtle (*Graptemys gibbonsi*) and the Pearl River Map turtle (*G. pearlensis*), whose cryptic speciation was not discovered until recently (Ennen, at al., 2010). Nevertheless, the presence of two distinct populations of *A. s. aspera* from neighboring drainages has important implications for the species as a whole. As this pattern of genetically distinct populations in various drainages is likely going to be prevalent throughout the species range. In a long-term scenario, genetic variability is a key factor in species persistence (Lande & Shannon, 1996).

While genetic differentiation was present on an inter-drainage scale, on smaller intra-drainage levels minimal genetic structure was found. Within the approximately 518 river km surveyed within the Pearl River drainage, only a single population was identified by STRUCTURE (Fig. 4.6). The Ross Barnett Dam, situated along the upper middle section of the Pearl River, serves as a barrier to up- and downstream movement in *G. oculifera* (Jones & Selman, 2009), and most likely restricts the movement of upstream and downstream *A. spinifera* as well. Previous studies have shown the presence of such barrier effects genetic connectivity in turtles (Santos, et al., 2016). However, *A. spinifera* turtles are a long-lived species, and can survive up to 50 years in the wild (Breckenridge, 1955). The Ross Barnett Dam was constructed relatively recently, in 1964 (Tipton, et al., 2004), therefore at most one generation has passed since its construction. This is not enough time for any observable barrier effects to present themselves (Landguth, et al., 2010). Similar studies of *Apalone* populations elsewhere (Reinertsen, et al., 2016), as well as *G. oculifera* in the Pearl River (Jones & Selman, 2009; Gillard, et al., 2015) also failed to detect the influence of a reservoir on population genetic structure. However, we would expect the dam to eventually have an impact on the population genetic structure of *A. spinifera* within the Pearl River drainage.

Apalone spinifera is a highly mobile (in water) species of riverine turtle and on average can move up to 141-122 meters per day with home ranges that can span an average of 1,750 m in males and 1,400 m in females (Ernst & Lovich, 2009). However, *G. flavimaculata*, the sister species of *G. oculifera* (Lamb et. al., 1994; Stephens & Wiens, 2003) that fills a similar ecological niche within the Pascagoula drainage, has similar home ranges of 1,800 m in males, and 1,500 m in females (Jones, 1996). A similar genetic study of the Pearl River endemic *G. oculifera* found patterns of isolation by distance (Gaillard, et al., 2015), this pattern was likewise present within the Pearl River *A. spinifera* populations (Fig. 4.5). The mobility of both species has likely led to the absence of strong intra-drainage structure. But, the positive correlation between genetic differentiation and geographic distance suggests that, while gene flow is taking place throughout the Pearl River, it is generally limited to geographically proximate locations.

The lack of strong genetic structure and no indication of isolation by distance for *A. spinifera* in the Pascagoula River was not expected given the highly dendritic nature of the river. Selman et al. (2013) found three genetically distinct populations of the Pascagoula River endemic *G. flavimaculata*. Population structure in *G. flavimaculata* has been attributed to an overall patchy distribution of populations, due to separated areas of suitable habitat, specifically adequate basking structure (Selman, et. al., 2013). *Apalone spinifera* on the other hand are considerably more of a generalist species that can occupy a wider array of habitats (Dreslik, et. al., 2005), and are known to bask not only on logs, rocks, or debris, but on sandbars or shore (Lindeman, 2001). Galoise et al. (2002) found that when there is limited suitable habitat *A. spinifera* has an increase in mobility, and individuals may inhabit different home ranges from year to year. The more generalist nature, and greater mobility of *A. spinifera* in instances of unsuitable habitat compared to *G. flavimaculata,* could be a possible explanation for their relatively panmictic population within the Pascagoula River drainage.

This is the first study of *A. spinifera* population genetics completed within the state of Mississippi. Overall, we found evidence, which supports inter-drainage structure, with two genetically distinct populations between the Pascagoula and Pearl River drainages. However, when intra-drainage structure is analyzed, both drainages seem to possess only a single population. To get a better understanding of the *A. spinifera* populations across the state, more samples should be collected from both the Pascagoula and the Pearl Rivers, to better inform analyses. The remaining Mississippi drainages will be sampled in the next few years, allowing us to observe if this pattern of inter-drainage genetic differentiation remains constant throughout every drainage within the state.

4.5 Tables

Table 4.1 River Drainage *Apalone spinifera* Genetic Summary Statistics

Population	**Locus**	**N**	**N_A**	**H_o**	**H_e**
Pearl	As12	43	8.000	0.674	0.556
	As13	44	9.000	0.795	0.720
	As15	44	5.000	0.727	0.748
	AsB07	44	4.000	0.409	0.449
	AsB08	43	4.000	0.395	0.645
	AsB12	44	6.000	0.364	0.373
	AsB14	43	4.000	0.651	0.581
	Mean	43.571	5.714	0.574	0.582
	SE	0.202	0.778	0.068	0.052
Pascagoula	As12	92	5.000	0.489	0.479
	As13	93	11.000	0.753	0.688
	As15	93	5.000	0.538	0.568
	AsB07	93	3.000	0.269	0.277
	AsB08	92	5.000	0.457	0.451
	AsB12	92	5.000	0.793	0.748
	AsB14	91	10.000	0.791	0.791
	Mean	92.286	6.286	0.584	0.572
	SE	0.286	1.128	0.076	0.070

Genetic summary statistics by locus including the sample size (N), number of alleles (NA), observed heterozygosity (Ho), and expected heterozygosity (He) for A. spinifera from the Pearl and Pascagoula drainages. Mean values and standard error (SE) are reported for each river.

Table 4.2 Pearl River Drainage *Apalone spinifera* Genetic Summary

Population	**Locus**	**N**	**N**$_A$	**H**$_O$	**H**$_E$
Upper Pearl	As12	6	3.000	0.667	0.500
	As13	6	4.000	0.500	0.681
	As15	6	4.000	0.500	0.708
	AsB07	6	3.000	0.833	0.611
	AsB08	6	3.000	0.333	0.569
	AsB12	6	3.000	0.500	0.403
	AsB14	6	3.000	0.500	0.569
	Mean	6.000	3.286	0.548	0.577
	SE	0.000	0.184	0.060	0.040
Reservoir Area	As12	18	4.000	0.611	0.465
	As13	19	7.000	1.000	0.729
	As15	19	5.000	0.895	0.741
	AsB07	19	4.000	0.474	0.536
	AsB08	19	4.000	0.579	0.602
	AsB12	19	4.000	0.474	0.497
	AsB14	19	4.000	0.842	0.626
	Mean	18.857	4.571	0.696	0.599
	SE	0.143	0.429	0.081	0.041
Middle Pearl	As12	9	6.000	0.778	0.679
	As13	9	4.000	0.778	0.562
	As15	9	4.000	0.778	0.698
	AsB07	9	3.000	0.444	0.438
	AsB08	9	4.000	0.222	0.673
	AsB12	9	2.000	0.111	0.105
	AsB14	8	2.000	0.500	0.469
	Mean	8.857	3.571	0.516	0.518
	SE	0.143	0.528	0.105	0.079
Lower Pearl	As12	9	5.000	0.667	0.568
	As13	9	5.000	0.556	0.679
	As15	9	4.000	0.556	0.660
	AsB07	9	1.000	0.000	0.000
	AsB08	8	3.000	0.250	0.555
	AsB12	9	4.000	0.333	0.296
	AsB14	9	3.000	0.444	0.370
	Mean	8.857	3.571	0.401	0.447
	SE	0.143	0.528	0.086	0.092

Statistics Genetic summary statistics by locus including the sample size (N), number of alleles (NA), observed heterozygosity (Ho), and expected heterozygosity (He) for A. spinifera from the Pearl River drainage. Mean values and standard error (SE) are reported for each pooled grouping.

Table 4.3 Pearl River Drainage Genetic and Geographic Distances

Pearl River Pairwise Population Fst Values				
Upper Pearl	Reservoir Area	Middle Pearl	Lower Pearl	
0.000	0.752	1.423	2.223	Upper Pearl
0.027	0.000	0.878	1.853	Reservoir Area
0.045	0.035	0.000	1.008	Middle Pearl
0.080	0.058	0.053	0.000	Lower Pearl

Genetic distance (FST) matrix between A. spinifera populations within the Pearl River drainage (below diagonal). River distance between sites based on GPS coordinates (above diagonal).

Table 4.4 Pascagoula River Drainage *Apalone spinifera* Genetic Summary Statistics

Population	Locus	N	N_A	H_O	H_E
Upper Bouie	As12	22	4.000	0.455	0.569
	As13	22	7.000	0.591	0.638
	As15	22	3.000	0.727	0.592
	AsB07	22	2.000	0.273	0.298
	AsB08	22	4.000	0.545	0.551
	AsB12	22	4.000	0.727	0.740
	AsB14	22	7.000	0.773	0.767
	Mean	22.000	4.429	0.584	0.593
	SE	0.000	0.719	0.068	0.058
Upper Leaf	As12	18	3.000	0.611	0.498
	As13	18	9.000	0.889	0.772
	As15	18	4.000	0.444	0.539
	AsB07	18	2.000	0.222	0.198
	AsB08	18	4.000	0.500	0.406
	AsB12	18	4.000	0.944	0.735
	AsB14	18	7.000	0.722	0.688
	Mean	18.000	4.714	0.619	0.548
	SE	0.000	0.918	0.097	0.077
Middle Leaf	As12	3	2.000	0.667	0.444
	As13	3	3.000	1.000	0.611
	As15	3	3.000	0.667	0.500
	AsB07	3	2.000	0.333	0.278
	AsB08	3	2.000	0.667	0.444
	AsB12	3	3.000	1.000	0.611
	AsB14	3	4.000	0.667	0.667
	Mean	3.000	2.714	0.714	0.508
	SE	0.000	0.286	0.087	0.051
Upper Chick	As12	18	4.000	0.500	0.410
	As13	19	5.000	0.789	0.648
	As15	19	3.000	0.421	0.553
	AsB07	19	3.000	0.263	0.237
	AsB08	18	4.000	0.444	0.366
	AsB12	18	5.000	0.778	0.741
	AsB14	17	8.000	0.706	0.763
	Mean	18.286	4.571	0.557	0.531
	SE	0.286	0.649	0.076	0.076

Table 4.4 (continued)

Middle Chick	As12	22	3.000	0.455	0.430
	As13	22	6.000	0.773	0.632
	As15	22	5.000	0.455	0.493
	AsB07	22	3.000	0.318	0.305
	AsB08	22	5.000	0.409	0.469
	AsB12	22	4.000	0.682	0.737
	AsB14	22	7.000	0.909	0.804
	Mean	22.000	4.714	0.571	0.553
	SE	0.000	0.565	0.082	0.067
Lower Chick	As12	7	4.000	0.429	0.459
	As13	7	4.000	0.714	0.684
	As15	7	3.000	0.714	0.653
	AsB07	7	2.000	0.000	0.245
	AsB08	7	2.000	0.286	0.408
	AsB12	7	4.000	0.857	0.745
	AsB14	7	5.000	0.857	0.776
	Mean	7.000	3.429	0.551	0.567
	SE	0.000	0.429	0.122	0.075
Pascagoula Proper	As12	2	1.000	0.000	0.000
	As13	2	2.000	0.500	0.375
	As15	2	3.000	0.500	0.625
	AsB07	2	3.000	1.000	0.625
	AsB08	2	1.000	0.000	0.000
	AsB12	2	3.000	1.000	0.625
	AsB14	2	3.000	1.000	0.625
	Mean	2.000	2.286	0.571	0.411
	SE	0.000	0.360	0.170	0.112

Genetic summary statistics by locus including the sample size (N), number of alleles (NA), observed heterozygosity (Ho), and expected heterozygosity (He) for A. spinifera from the Pascagoula River drainage. Mean values and standard error (SE) are reported for each pooled grouping.

Table 4.5 Pascagoula River Drainage Genetic and Geographic Distances

Pascagoula River Drainage Pairwise Population Fst Values					
Upper Bouie	Upper Leaf	Upper Chick	Middle Chick	Lower Chick	
0.000	44.5	332.0	192.0	135.0	Upper Bouie
0.018	0.000	363.0	231.0	177.0	Upper Leaf
0.016	0.007	0.000	134.0	192.0	Upper Chick
0.011	0.018	0.014	0.000	57.0	Middle Chick
0.013	0.022	0.016	0.015	0.000	Lower Chick

Genetic distance (Fst) matrix between A. spinifera populations within the Pascagoula River drainage (below diagonal). River kilometer between sites (above diagonal).

4.6 Figures

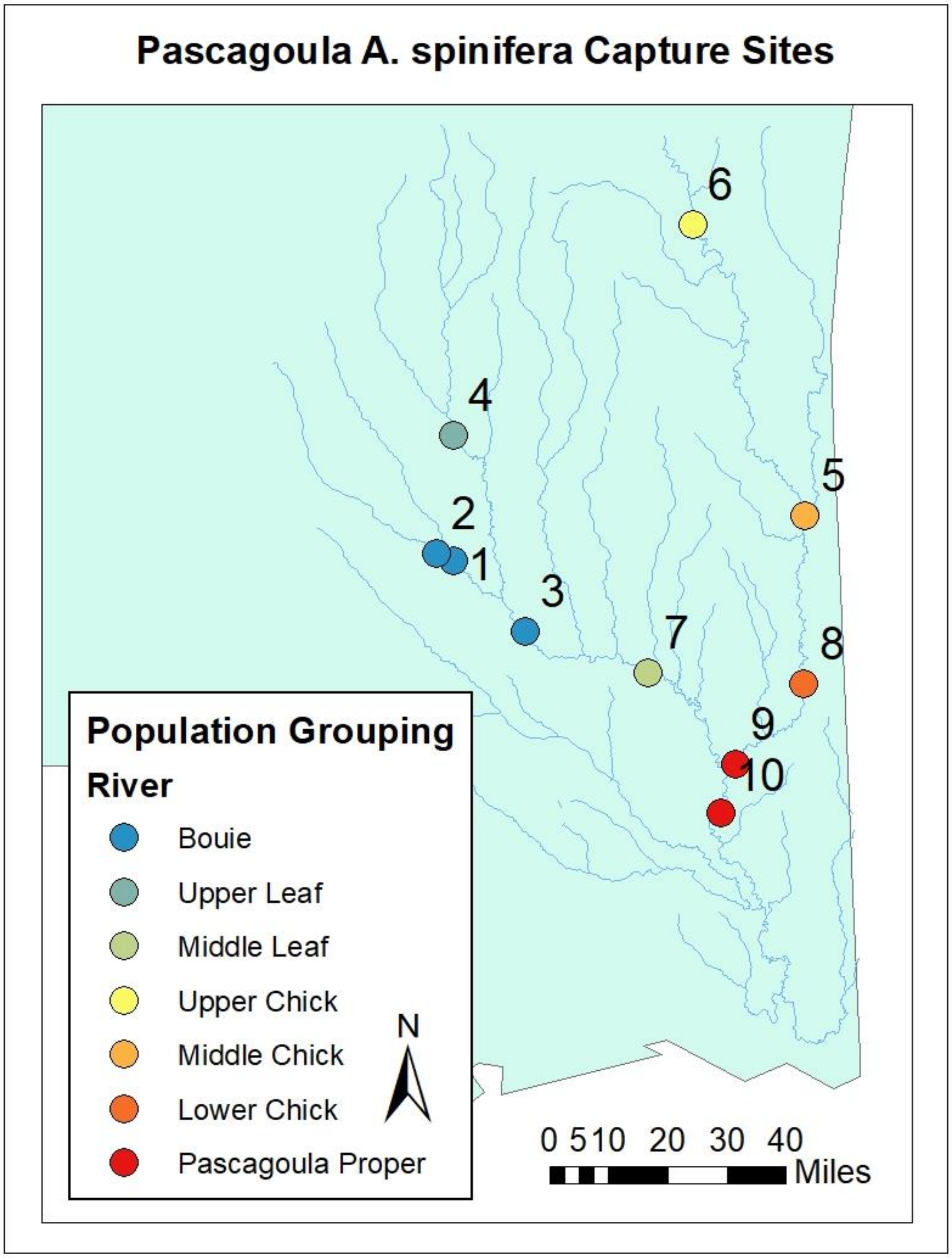

Figure 4.1 *Apalone spinifera* capture sites within the Pascagoula River Drainage.

These sites have been grouped into 7 populations to perform genetic analyses. Bouie Population: 1) Upper Bouie, 2) Murchinson Lake, 3) Wedgeworth. Pascagoula Proper Population: 9) Charles Deaton, 10) Pascagoula WMA. The remaining 5 populations are all in from distinct sites: Upper Leaf (4), Middle Leaf (7), Upper Chick (6), Middle Chick (5), and Lower Chick (8).

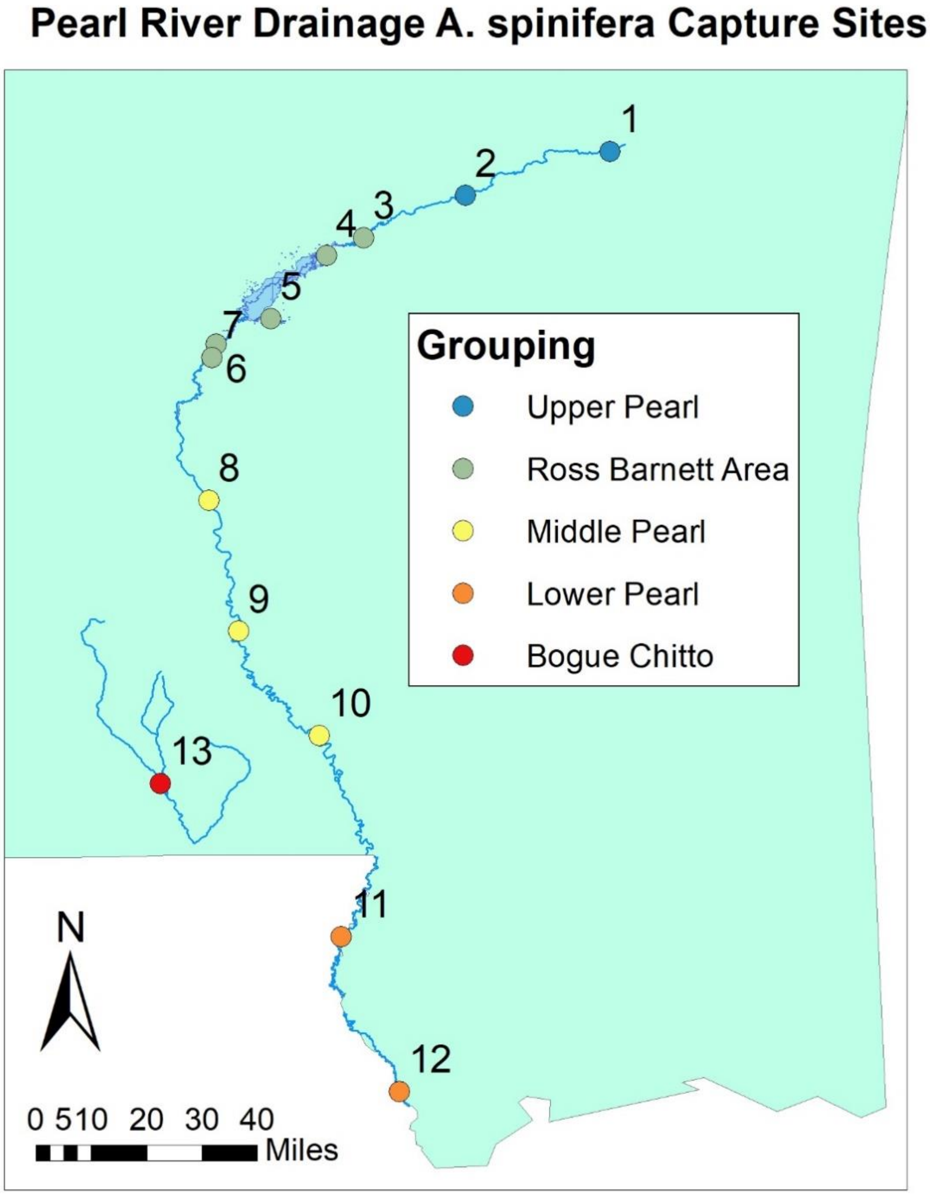

Figure 4.2 *Apalone spinifera* capture sites within the Pearl River Drainage.

These sites have been grouped into 5 populations to perform genetic analyses. Upper Pearl population: 1) Philadelphia and 2) Carthage. Reservoir Area population: 3) Coal Bluff, 4) Ross Barnett North, 5) Ross Barnett South, 6) LeFleur's Bluff, and 7) Crystal Lake. Middle Pearl population: 8) Georgetown, 9) Atwood, and 10) Columbia. Lower Pearl population: 11) Bogalusa and 12) Stennis. Bogue Chitto population: 13) Bogue Chitto.

Figure 4.3 Pearl and Pascagoula River Drainage Percent Ancestry

Bar plots of membership coefficients for K=2 showing two groups comprised of A. spinifera from the Pearl River (1) and Pascagoula River (2).

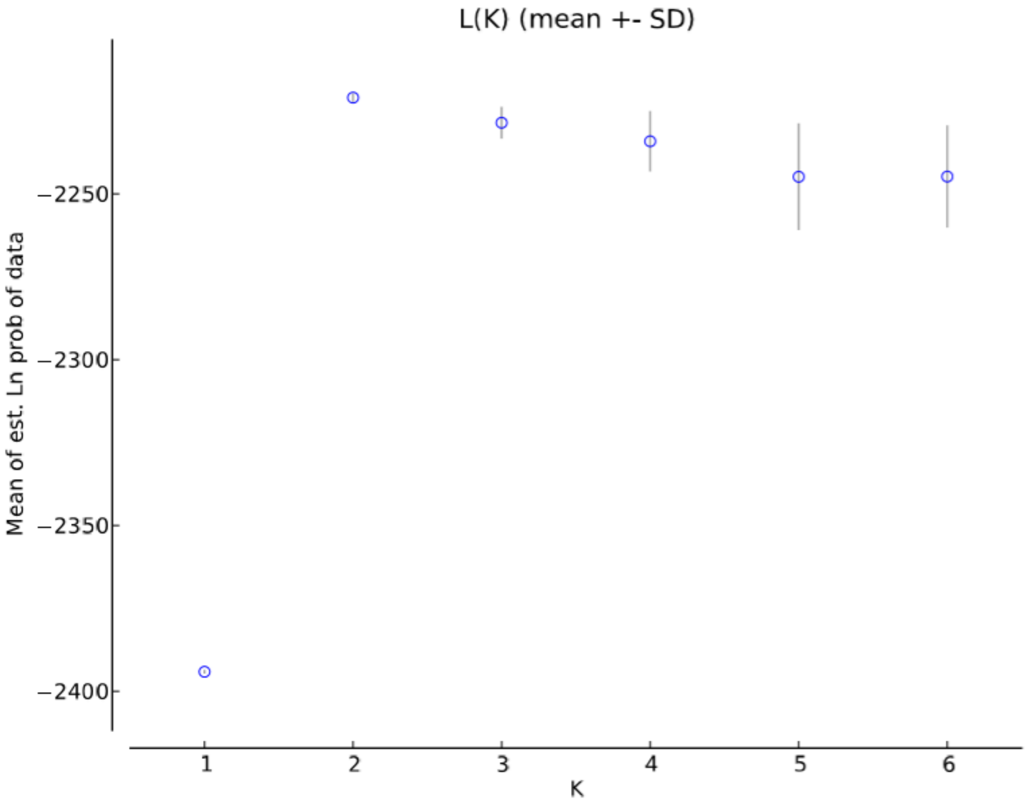

Figure 4.4 Inter-drainage Mean Likelihood Plot

The plot of the mean likelihood scores from the A. spinifera inter-drainage comparisons STRUCTURE analysis. This plot shows the analysis most supported K of 2.

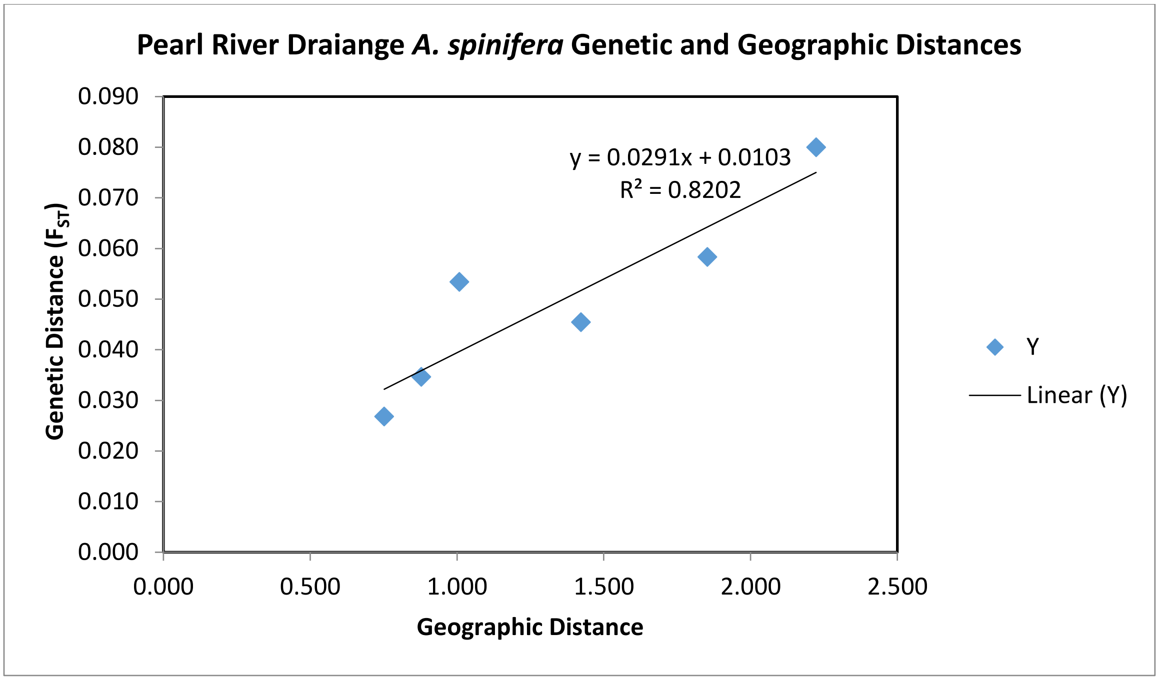

Figure 4.5 Pearl River Drainage Mantel Test

A Mantel test of isolation by distance of the Pearl River A. spinifera populations sampled. This test supports that isolation by distance is taking place within the Pearl River drainage (r = 0.906, P = 0.044).

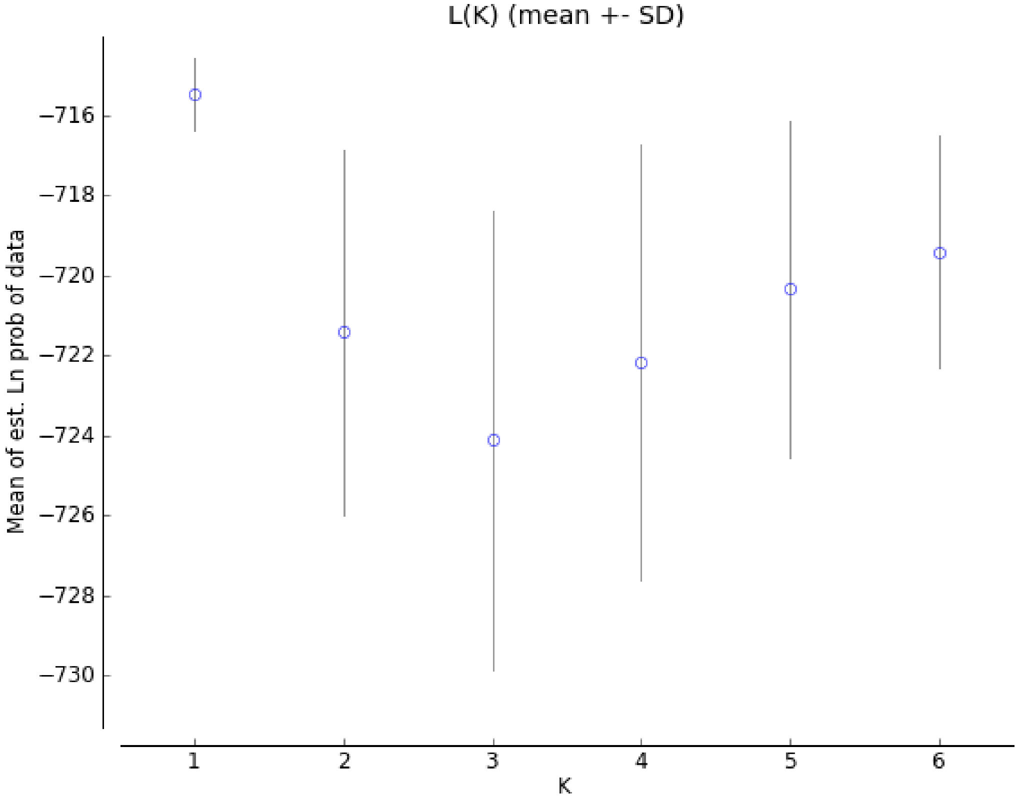

Figure 4.6 Pearl River Drainage Mean Likelihood Plot

The plot of the mean likelihood scores from the A. spinifera Pearl River drainage comparisons STRUCTURE analysis. This plot shows the analysis most supported K of 1.

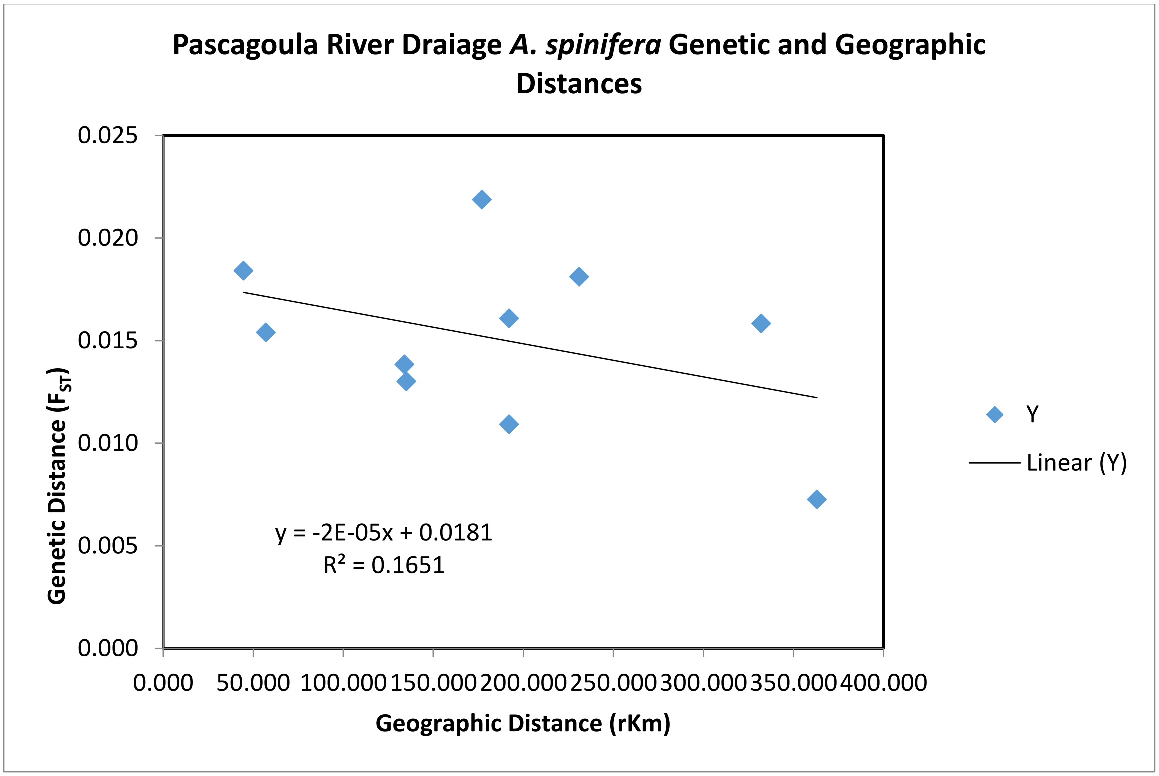

Figure 4.7 Pascagoula River Drainage Mantel Test

A Mantel test Pascagoula River A. spinifera populations sampled. This test does not support that isolation by distance is taking place within the Pascagoula River drainage ($r = 0.165$, $P = 0.11$).

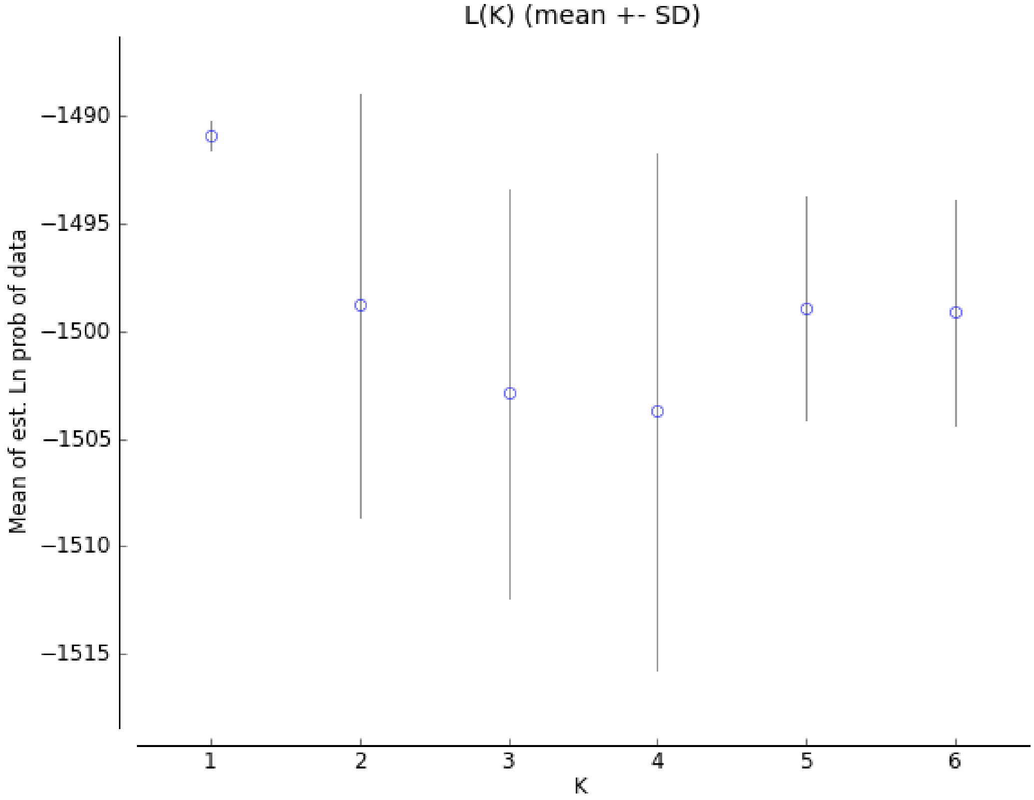

Figure 4.8 Pascagoula River Drainage Mean Likelihood Plot

The plot of the mean likelihood scores from the A. spinifera Pascagoula River drainage comparisons STRUCTURE analysis. This plot shows the analysis most supported K of 1.

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