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Reproductive Timing in Percina aurora and other Percina species in the Pascagoula River Watershed

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Reproductive Timing in *Percina aurora* and other *Percina* species in the Pascagoula River Watershed

by

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ABSTRACT

The Southeastern United States is home to high levels of aquatic biodiversity and was recently named one of the Earth's biodiversity hotspots. Within this region, growing human populations continue to place pressure on aquatic ecosystems and the resident species. Darters (Percidae: *Percina*) represent a large proportion of the freshwater fish diversity in the region. However, for some species the fundamentals of age, growth, reproductive timing and effort, and ecology remain poorly understood. That said, understanding the timing, duration, and effort allocated to reproduction is vital to successful management of any species. The pearl darter, *Percina aurora*, has been recently listed as threatened due to its contracting range, likely due to anthropogenic pressure on the Pearl and Pascagoula River Systems. This species has been extirpated from the Pearl River, cutting its known distribution in half. Overall, little is known of pearl darter life history as surprisingly few reproductive adults have been captured. Thus, there remains a major knowledge gap in terms of the life history and reproductive ecology of this species.

The purpose of this project was to examine the age, growth, and reproductive ecology of pearl darters (*Percina aurora*) and other coexisting *Percina* species in the Pascagoula River Basin. Four *Percina* species were collected and the gonadosomatic index (GSI) was calculated over time to better understand the reproductive effort and timing. The *Percina* species collected included *P. aurora*, *P. vigil*, *P. sciera*, and *P. suttkusi*. The goal was to find the spawning window of each species as there may be some overlap with the pearl darter. This overlap could lead to larval competition for resources, placing another stressor on the threatened pearl darter. To better understand the age and

growth of this species, growth was modeled using the Von Bertalanffy Growth Model (VBGM) based on size measurements of previously collected and vouchered pearl darter specimens throughout the range. Lastly, future directions of the research on the life history of the pearl darter were discussed.

Keywords: Pascagoula River basin, Percina, Reproduction, Life History, Threatened Species

DEDICATION

To my grandparents and uncle, who sparked my interest in biology and have always supported my dreams.

ACKNOWLEDGMENTS

I would like to thank Dr. Schaefer for his guidance and support throughout this process. I would not have been able to complete this without his guidance and patience. I would also like to acknowledge Noah Daun, Scott Clark, Brian Kreiser, Langston Haden, and Loren Stearman in all their help in collections. Furthermore, I want to acknowledge the U.S. Fish and Wildlife Service for their generous funding of this project and the Mississippi Museum of Natural Science for loaning *Percina aurora* specimens.

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LIST OF ABBREVIATIONS

GSI	Gonadosomatic Index
SL	Standard Length
TLHM	Trilateral Life History Model
US	United States of America
USM	The University of Southern Mississippi
VBGF	Von Bertalanffy Growth Function
VBGM	Von Bertalanaffy Growth Model

CHAPTER I: INTRODUCTION

Biodiversity is an extremely important factor in any ecosystem. It is vital to maintaining a stable ecosystem and governs the magnitude and efficiency in providing various ecosystem services (Gamfeldt *et al.,* 2008). Furthermore, biodiversity has economic value as it provides goods to human society (Gamfeldt *et al.,* 2008). A decrease in biodiversity, through extirpation of resident species, can destabilize an ecosystem as there may not be another species present to fill the resident species' niche.

Freshwater is one of the most diverse ecosystems and has over 10,000 described fish species which make up 40% of global fish diversity (Dudgeon *et al*., 2006). Within the Southeastern United States (US) there are high levels of aquatic biodiversity, where the Southeastern US was recently named one of the Earth's biodiversity hotspots (Dudgeon *et al.,* 2006). Freshwater resources are valuable and in high demand, placing them at risk from increasing anthropogenic pressures. The increasing anthropogenic pressures have resulted in some of the most diverse freshwater ecosystems, and the species residing in them, becoming more imperiled than any other system. For instance, freshwater mussels are the most imperiled taxa in North America (Garner, 1999). Within freshwaters, small headwater streams are more prone to land use changes and a variety of anthropogenic pressures, leaving those species residing in these smaller headwater streams at higher risk of extinction. Amongst the many freshwater fish, Darters (Percidae: *Percina*) represent a large proportion of the freshwater fish diversity in the region.

Protection and management of any species of conservation concern requires an understanding of the timing, duration, and effort allocated to reproduction. Within the Southeastern US, growing human populations continue to place pressure on aquatic

ecosystems and the resident species. For instance, nontraditional natural gas extractions in Cypress Creek, Arkansas, have led to habitat degradation of the redfin darter (*Etheostoma whipplei*), which has a very restricted range (Stearman *et al.,* 2014).

However, for some species the fundamentals of age, growth, reproductive timing and effort and their ecology remain poorly understood. Conducting research on the life history of a species is a crucial part of understanding how to better protect them. In particular, understanding the spawning time of a fish may help determine when to limit boating and other recreational activities in known spawning regions. Documenting where spawning occurs may also help in protecting the habitats needed to complete a life cycle. Life history research can also help determine the probability of recovery or survival of a species after major environmental changes. This survival is privy to whether a species is a specialist or a generalist. A generalist species will be capable of thriving in varying habitats and resources, while a specialist will be restricted to surviving in a specific habitat with special resources. A generalist species will be more likely to survive major changes to its ecosystem than a specialist species (Grime, 1977).

Furthermore, some of these life history traits have been correlated to the environment the species resides in. The trilateral life history model (TLHM) is a trait-environment model that summarizes expected trade-offs between juvenile survival, fecundity, and generation time (Bennett *et al.,* 2016). Though not represented in this study, there are three major strategies that a species may use to increase fitness: opportunistic, equilibrium, and periodic. The opportunistic strategy is favored in highly variable environments as it shortens generation time and population growth rate. The equilibrium strategy is favored under stable environmental conditions which maximizes

juvenile survival with large egg size or parental care. Lastly, the periodic strategy is favored under predictably variable conditions, so fecundity is maximized by delaying reproduction until a large egg size is reached (Bennet *et al.,* 2016). By observing the stability of the environment, the Gonadosomatic Index (GSI) and other life history traits can be sufficiently predicted in a fish species based on the TLHM (Bennet *et al., 2016*).

Study Species

Percina aurora

The pearl darter, *Percina aurora,* was formally described in 1994 (Suttkus *et al.,* 1994) and is now listed as endangered in the state of Mississippi and threatened under the US Endangered Species Act (Clark *et al.,* 2018). Considering its fairly recent recognition, relatively little is known about the fundamentals of age, growth, reproductive timing and effort, and ecology. This knowledge gap may prevent effective management of this species. What is known from non-reproductive period sampling is that the favored adult habitat is deeper and slower flowing water with finer substrates and loose detrital accumulation (Clark *et al.,* 2018). There are few documented collections during putative spawning periods, but spawning habitat is postulated to happen over shallower and faster flowing sand bars with more gravel and sand substrates (Clark *et al.,* 2018).

The major reason for the listing of the pearl darter is that this species was extirpated from the Pearl River drainage, which reduced its known range by 50% (Tipton *et al*., 2004). In the remaining range of the Pascagoula River drainage, the pearl darter is found in low abundance, and with a changing environment due to climate change and human factors, it is critical that more information is known on the pearl darter to protect it from potential extinction (Tipton *et al.,* 2004). One way to gain more information on

the pearl darter is to compare the pearl darter to other species within the same genus *Percina* residing in similar habitats, as closely related species may give clues into the potential life history of the pearl darter.

Percina vigil

The saddleback darter, *Percina vigil*, is found in sand and gravel raceways in streams of the Gulf Coastal Plain and Mississippi River embayment (Heins & Baker, 1989). *Percina vigil* is one of the smallest species among the genus and is believed to live no more than two years and reach sexual maturity at one year of age (Heins & Baker, 1989). Reproduction is thought to occur from mid-February to April, but individuals can spawn multiple times during the reproductive season (Heins & Baker, 1989). This was determined through measuring the stages of the developing oocytes within the females. The result found that some females had oocytes in all four stages of reproductive condition which is what led to the determination that individuals could spawn multiple times during the reproductive season (Heins & Baker, 1989).

Percina sciera

The dusky darter, *Percina sciera*, is found in the Eastern, Southern, and Southeastern US in small and large rivers and streams and is one of the most abundant darters (Page & Smith, 1970). This species is most abundant in habitats with fast-moving currents over gravel bottoms and depth greater than one foot, but as a generalist species, the dusky darter can occur in other habitats (Page & Smith, 1970). *Percina sciera* darkens slightly as breeding season approaches, which may start as late as April (Page & Smith, 1970). Furthermore, both sexes are sexually mature and can spawn at one year of age, though their spawning time can vary (Page & Smith, 1970). Peak spawning is thought to

occur between late May and early June (Page & Smith 1970). In comparison to other studied darter species, *Percina sciera* is more closely related to *Percina nigrofasciata,* the blackbanded darter, as they reside in the subgenus *Hadropterus. Percina vigil* and *Percina aurora* belong to the subgenus *Imostoma* and subgenus *Cottogaster*, respectively (Near *et al.,* 2011).

Percina suttkusi

The gulf logperch, *Percina suttkusi*, is found in the western tributaries of Lake Pontchartrain and eastward through the Pearl, Pascagoula, and Mobile River basins in Mississippi and Alabama (Thompson, 1997). *Percina suttkusi* can survive in multiple different habitats but appear to prefer larger mainstem streams, such as the main channel of the Pearl, Pascagoula, and Tombigbee Rivers (Thompson, 1997). *Percina suttkusi* spawns from January to March as batch spawners, where one individual can have multiple spawning events (Thompson, 1997). The juveniles have been found closer to the shallow shoreline in mid-April to mid-May (Thompson, 1997).

Percina nigrofasciata

The blackbanded darter, *Percina nigrofasciata,* is abundant in small streams of the Eastern Gulf and Southern Atlantic Coastal Plain (Mathur, 1973). This species resides in a variety of stream habitats but specializes in silty streams with sand or gravel (Mathur, 1973). Reproduction varies across the range, but for the Louisiana and Mississippi region, spawning is thought to begin in mid-February and stop in April (Hughey *et al*., 2021). Clutch sizes also vary based on locality, with Florida having the smallest reported clutch sizes (Hughey *et al.,* 2021).

Overall, determining the life history of any species is crucial in further understanding how best to protect the species. The pearl darter, *Percina aurora*, is listed as threatened in the US Endangered Species Act and endangered in the state of Mississippi (Clark *et al.,* 2019). A major aspect of life history is reproduction, and if reproduction is known for a species, that species can be better protected. Thus, the purpose of this project was to examine the age, growth, and reproductive ecology of pearl darters (*Percina aurora*) and other coexisting *Percina* species in the Pascagoula River basin. The saddleback darter (*Percina vigil*), dusky darter (*Percina sciera*), and the gulf logperch (*Percina suttkusi*) were also collected to determine if there was spawning overlap that could lead to larval competition for resources with the pearl darter that could place another stressor on the pearl darter. Furthermore, to better understand the lifespan and growth of this species, growth was modeled using the Von Bertalanffy Growth Function (VBGF) which is based on size measurements of previously collected museum specimens throughout the range (Fabens, 1965). The VBGF is a standard statistical method that portrays small juveniles captured in spring as being born that year. These juveniles are then considered to be year one fish. Over the year, juveniles will become larger in size before entering reproductive adulthood (Fabens, 1965). Once a reproductive adult, the individual is considered a year two fish (Fabens, 1965).

CHAPTER II: METHODS

Specimens of all species were collected from the Pascagoula River Basin of Southeastern Mississippi. Reproductive adults of four *Percina* species (*Percina aurora, Percina vigil, Percina sciera,* and *Percina suttkusi*) were sampled from the Bouie River north of Hattiesburg (Hwy 11 access, 31.35433, - 89.2833) with weekly seining of shallow riffles and gravel bar habitat between February and June of 2021. Outside of the putative reproductive period, pearl darters were collected throughout the Chickasawhay, Leaf, and Pascagoula Rivers as part of a regular monitoring program. Seining in those larger river habitats was conducted on large sand bars, typically near detrital accumulations at the upper or lower end of the sand bars. All sampling used a 6.1 × 1.8 m heavy-leaded seine with 3.2 mm mesh. This type of seine is designed to specifically target benthic species (the extra lead disturbs the sediment) that may not be as efficiently sampled with a standard seine. Seining was conducted by pulling upstream, generally parallel to shore, before being brought into shore. All other species besides the target genus, *Percina*, were released. The specimens collected during the reproductive period were fixed in 10% formalin until they could be processed in the laboratory. The specimens collected throughout the basin outside of the reproductive period were fixed in formalin for approximately two weeks and then transferred to 70% ethanol to be preserved. Because ethanol dehydrates tissues and alters body condition, GSI data were only collected on formalin fixed specimens. Modeling of growth used ethanol preserved specimens.

To determine reproductive effort, each specimen was measured (standard length [SL], and wet mass) before the gonads and the gastrointestinal tract were removed. The

sex, gonad mass, and eviscerated carcass mass were then recorded. The GSI was calculated for each specimen by dividing the gonad mass by the wet mass. This value was then multiplied by 100 to give a percentage.

Statistical Methods

Spawning windows (periods over which spawning takes place) for each species were inferred after inspecting plots of GSI over time. A computer generated smooth curve was then added that most closely followed the data pattern to determine estimated spawn time. The spawning windows were also aligned with the projected birth from the growth curve. For this sampling year, *Percina suttkusi* was not used in the comparison as there were not enough specimens captured during 2021 sampling efforts to adquatly graph their GIS data. For literature comparison, the GSI values of *Percina aurora* were compared to previous literature data for the saddleback darter (*Percina vigil*), the dusky darter (*Percina sciera*), the gulf logperch (*Percina suttkusi*), and the blackbanded darter (*Percina nigrofasciata*) (Heins & Baker, 1989; Page & Smith, 1970; Hughey *et al*., 2021; Thompson, 1997).

Modeling Growth

To model growth, the SL (mm) of all vouchered specimens in The Univeristy of Southern Mississippi (USM) and Mississippi Museum of Natural Science collections were taken. This included 847 individuals that were fixed in formalin and then preserved in 70% ethanol. An estimated 497 of these specimens came from USM and an estimated 350 specimens from the Mississippi Museum of Natural Science. Preliminary analyses made it clear that pearl darter size decreased with increasing stream size. To avoid

biasing growth models, the Pascagoula River (mainstem) and the Leaf and Chickasawhay Rivers (two major tributaries) were all modeled separately.

For the establishment of the VBGM, the pearl darter adults collected in the spring are considered to be year two fish and are known to be offspring from year one spawning, considering the lifespan of the pearl darter. To create the growth curve, the parameters of Kirkwood (1983) were used. These parameters included the assumptions that first year individuals are juevniles allocating their resources to growth and not reproduction and first year spawners are allocating resources to reproduction and not growth (Kirkwood, 1983).

CHAPTER III: RESULTS

General Efforts

From February 24, 2021 to June 30, 2021, 33 male and 15 female *Percina aurora* were collected. In the same time frame, 44 male and 34 female *Percina sciera*, 59 male and 44 female *Percina vigil*, and 5 male and 3 female *Percina suttkusi* were caught. A total of 242 fish were collected for the analysis of reproductive timing and effort (Table 1). The sex ratio for all species collected was male biased, particularly early in the sampling period (Table 2).

Table 1. Average number of males and females of four *Percina* species. Sampling took place from February 24, 2021 to June 30, 2021

Collection	Date	*Percina aurora*		*Percina sciera*		*Percina vigil*		*Percina suttkusi*	
		Male	Female	Male	Female	Male	Female	Male	Female
PD21-01	2/24/21	2	1	0	0	0	0	0	0
PD21-02	2/24/21	3	0	6	4	0	3	0	0
PD21-03	3/5/21	1	0	3	2	12	0	0	0
PD21-04	3/5/21	0	0	11	4	4	14	0	0
PD21-05	3/10/21	0	1	2	0	5	1	0	0
PD21-06	3/10/21	2	1	0	1	5	3	0	0
PD21-07	3/10/21	0	0	0	4	4	4	0	0
PD21-08	3/15/21	4	0	2	1	5	5	0	0
PD21-09	3/15/21	2	0	4	3	10	1	1	0
PD21-10	3/15/21	0	0	6	3	3	9	0	0
PD21-11	3/22/21	0	0	1	1	2	0	0	0
PD21-12	4/6/21	5	0	1	0	2	0	2	0
PD21-13	4/6/21	0	0	0	1	4	2	0	0
PD21-14	4/6/21	0	0	3	1	1	1	0	0
PD21-15	4/30/21	2	0	0	0	0	0	0	0
PD21-16	4/30/21	2	0	0	0	0	0	0	0
PD21-17	5/19/21	2	3	0	5	0	1	0	0
PD21-18	5/19/21	1	1	0	2	0	0	1	2
PD21-19	5/23/21	3	2	3	0	1	0	0	0
PD21-20	5/23/21	0	0	1	0	0	0	0	0
PD21-21	6/30/21	1	0	1	0	0	0	1	1

Table 1 (continued).

PD21-22	6/29/21	1	3	0	0	0	0	0	0
PD21-23	6/30/21	1	2	0	0	0	0	0	0
PD21-24	6/30/21	1	1	0	1	1	0	0	0
	Total	33	15	44	34	59	44	5	3

Table 2. Average number of males and females per month of *Percina* species in 2021

Month	*Percina aurora*		*Percina sciera*		*Percina vigil*		*Percina suttkusi*	
	Male	Female	Male	Female	Male	Female	Male	Female
February	5	1	6	4	0	3	0	0
March	9	2	29	19	50	37	1	0
April	9	0	4	2	7	3	2	0
May	6	6	4	8	1	1	1	2
June	4	6	1	1	1	0	1	1

Reproductive effort (GSI) for pearl darter males and females seemed to peak in late March and early April, declining rapidly by late June (Figure 1). Mean GSI values during peak periods were 0.514 (± 0.087 SE) for males and 2.197 (± 0.527 SE) for females (Figures 3, 4). It should be noted that GSI values were clearly increasing in our February sampling, but it is difficult to conclude the significance as Februrary through April samples were strongly male biased (Table 2). No females were captured in April, when male GSI values peaked (Figure 1), suggesting females and males are using different habitats during this period. Spawning most likely ends by June, as the lowest GSI values were found for that month in both males and females. Male pearl darters had a mean low GSI of 0.095 (± 0.017 SE) and females had a mean GSI of 0.498 (± 0.026 SE) (Figures 3, 4).

Percina vigil spawned earlier, having the highest male and female GSI in late February or early March. Males had the highest value in March with an average GSI of

0.519 (± 0.031 SE) (Figure 3). While females had the highest GSI values in February with a GSI average of 7.746 (± 0.404 SE) (Figure 4). GSI values then declined, suggesting a spawning window from February through mid-March and an end to spawning in May. The lowest GSI values for *Percina vigil* males were in May with one male having a GSI of 0.197 (Figure 3). The one female captured in May also had the lowest GSI value of 0.481 (Figure 4). However, there was one male *Percina vigil* caught in June with a GSI value of 0.240 (Figure 3). Although there did not seem to be a strong sex ratio bias in any months, there were slightly more males caught than females (Table 1).

GSI values in *Percina sciera* seemed to peak in mid-May, though the pattern was weak and sample sizes in April-June were small (Figure 2). The highest GSI peak in males was 0.313 in June with only one captured specimen, while in female *Percina sciera* the highest average GSI was found to be 5.632 (± 1.310 SE) in April (Figure 4). Spawning appeared to end in May, with the male captured in June potentially being a late spawner. The female *Percina sciera* GSI values peaked in April before decreasing in May with the lowest GSI value of 3.141 of one collected specimen (Figure 4).

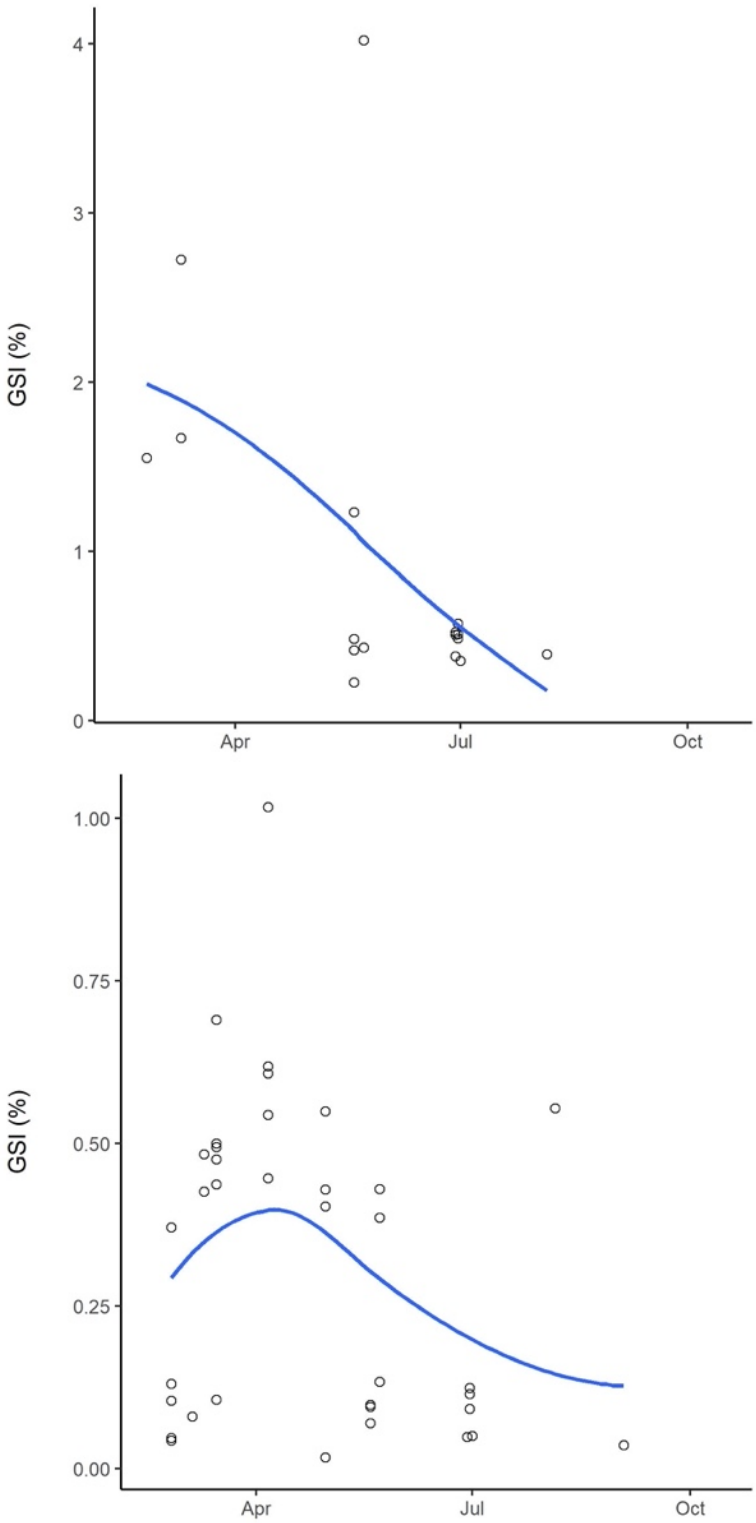

Figure 1. Percina aurora GSI values between Feburary 2021 and June 2021; values for females are shown on the upper graph and for males on the lower graph

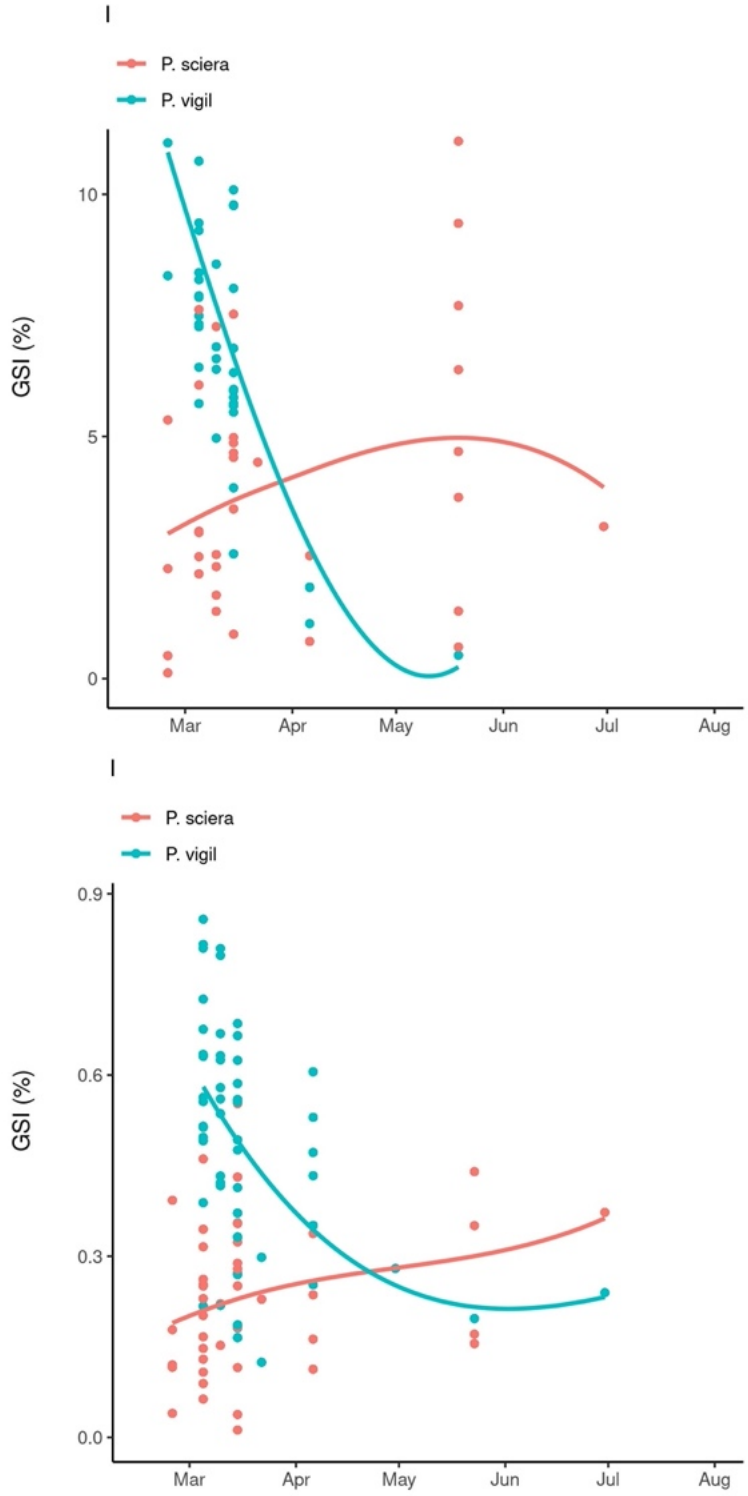

Figure 2. Percina sciera and Percina vigil GSI values between Feburary 2021 and June 2021; values for females are on the upper graph and for males on the lower panel

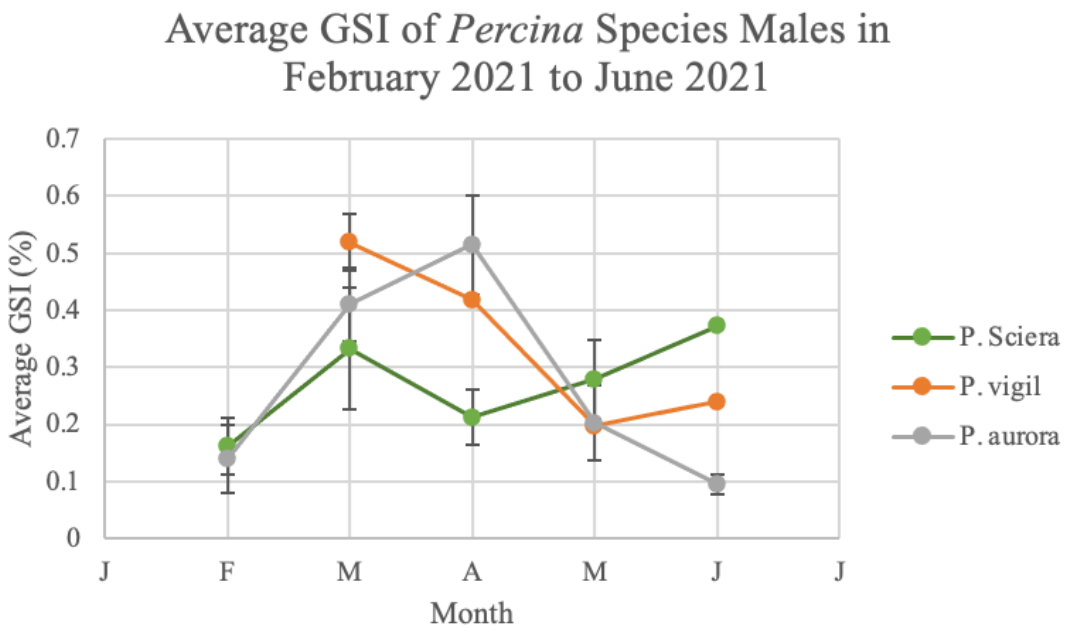

Figure 3. Monthly averages of GSI percent of Percina sciera, Percina vigil, and Percina aurora males in Feburary 2021 and June 2021

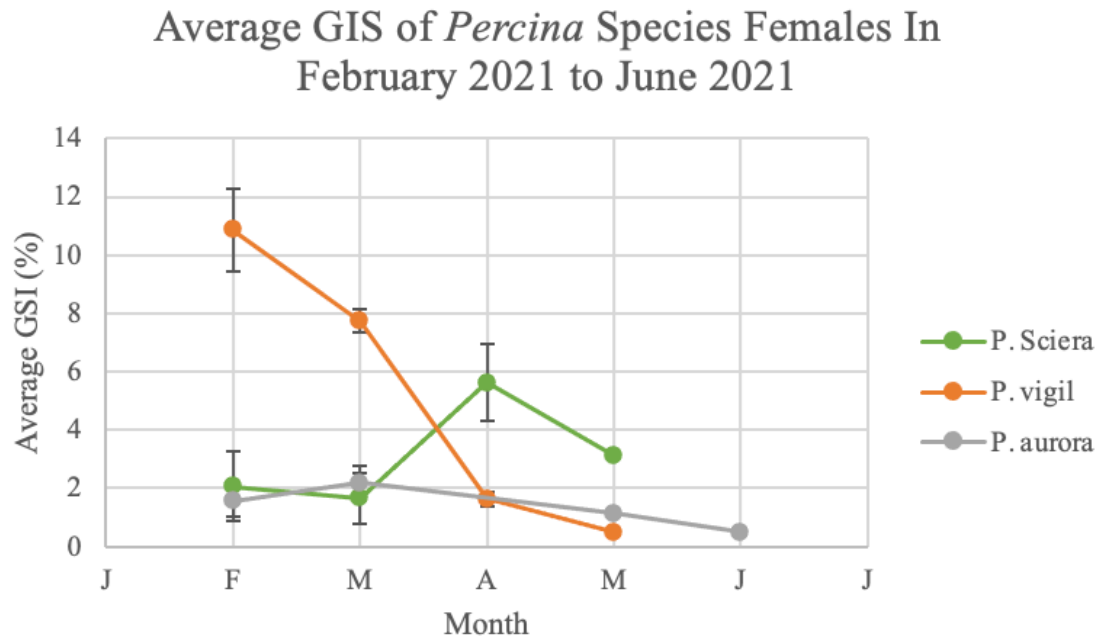

Figure 4. Montly averages of GSI percent of Percina sciera, Percina vigil, and Percina aurora females in Feburary 2021 and June 2021

Growth Curve

The VBGM suggests Pearl Darters hatch in late March or early April and grow to a size of approximately 38 - 45 mm SL at the end of their first year (Figure 5). The modeled hatch time from growth models aligns with the GSI data collected in 2021 as spawning is assumed to take place in March and April (Figure 1). Age one adults are reproductive, and models suggest limited growth over the second year when energy is likely shunted to reproductive tissues. There are few records suggesting a third-year class with maximum sizes of 48.6 mm in the Pascagoula River, 55 mm in the Leaf River, and 56.3 mm in the Chickasawhay River (Figure 5).

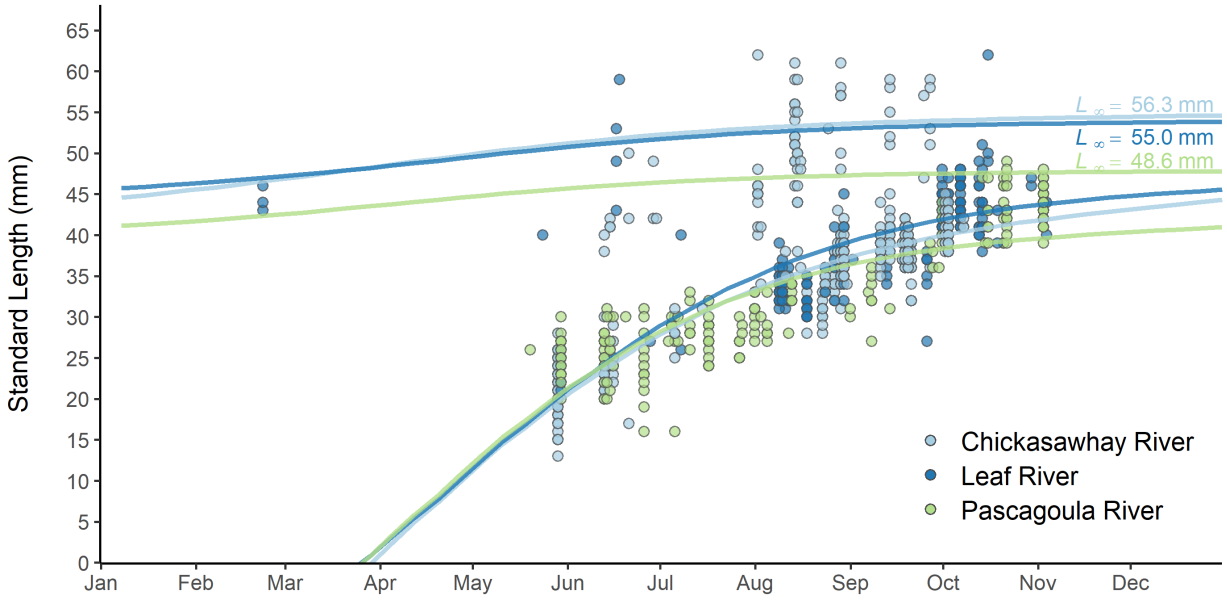

Figure 5. Von Bertalanffy Growth Curve of Percina aurora from 2020 to 2021

CHAPTER IV: DISCUSSION

The Gondasomatic Index data and growth models both suggest peak reproduction for pearl darters in the Pascagoula River basin is late March or early April. The decline in GSI after that time suggests a fairly short spawning window that falls between that of *Percina vigil* and *Percina sciera* in this system. Overall, it appears that both males and females begin preparation for spawning in January – February. Future research will try to identify habitat use and GSI during this crucial period. For the year 2021, the short spawning window, combined with the relatively low GSI values, is curious as these two factors could result in a fairly low capacity for population growth. In fact, pearl darters are not known for high local abundance. Rather, they have historically been found in small numbers throughout their range (Clark *et al.*, 2018).

Gonadsomatic Index Value Comparison

Pearl darter females have relatively low GSI values compared to other *Percina* species at their peak spawning. The average GSI value for *Percina aurora* was 2.179 (± 0.527 SE), while *Percina vigil* females had a peak value of 7.746 (± 0.404 SE). Lastly, *Percina sciera* females had a peak GSI value of 5.632 (± 1.310 SE). Though not found during this study, the peak GSI value for *Percina nigrofasciata* females is estimated to be 10.5 (Wieland, 1983). This study could be underestimating GSI of *Percina aurora,* considering relatively few (n=3) females were sampled during what we considered to be peak spawning period.

Sex Ratio Comparison

The skewed sex ratios during our sampling were not expected and could also be biasing results. It seems likely that the biased sex ratio could be a result of males and

females using different habitats when acquiring resources versus spawning. While the mating system of pearl darters is not well understood, females may be moving into a spawning habitat that is already occupied by males. It has been recorded that other *Percina* species move to another habitat for spawning. The frecklebelly darter, *Percina stictogaster*, has been documented to move from its usual low-velocity habitat adjacent to riffles to faster water during and just before spawning (Eisenhour *et al.,* 2013). Although the frecklebelly darter sex ratio was consistently 1:1, unlike that of the pearl darter (Eisenhour *et al.,* 2013). In contrast, both *Percina nigrofasciata* and *Percina sciera* are generalists, so a change in habitat for spawning has not been documented (Mathur, 1973; Page & Smith, 1970).

Spawning Window Comparison

In comparison to other coexisting *Percina* species, the spawning for *Percina aurora* appears to fall between the windows for *Percina vigil* and *Percina sciera* in this system. Though *Perica vigil* spawning can occur as late as early May, this study found that it likely peaked in February or March of 2021 (Heins & Baker, 1989). These timings are consistent for this species studied in other systems (Heins & Baker 1989; Page & Smith, 1970). The differences in timing should alleviate the potential for larval competition and will aid in efforts to quantify recruitment through ongoing larval sampling work.

Although not surveyed in 2021 sampling, the gulf logperch, *Percina suttkusi,* and the blackbanded darter, *Percina nigrofasciata*, are found in the same river system as the pearl darter and are thought to have some interaction with the species. Thus, the spawning windows of these two species indicate whether their larvae may compete with that of the pearl darter. *Percina suttkusi* has a spawning time between January and March, and one

individual can spawn multiple times within the season (Thompson, 1997). Compared to the pearl darter, this window is before their April peak spawning, which seems to alleviate larval competition. The adults are found in a similar habitat as the pearl darter, but they have a broader range of habitats, suggesting they will not be competing with the pearl darter in terms of resources (Thompson, 1997). Similarly, the blackbanded darter, *Percina nigrofasciata,* is found in a different habitat than the pearl darter but does reside in the same tributary. In the Southeastern US, *Percina nigrofasciata* spawning will take place mid-February and is expected to end by April when the pearl darter reaches peak spawning (Hughey *et al.*, 2021). *Percina nigrofasciata* are also considered a more generalist species and do not have a documented spawning habitat (Mathur, 1973).

Von Bertalanffy Growth Model

Growth models were consistent with hatching occurring just after the observed peak GSI values. The model suggested pearl darters reach 45 mm in their first year, reach sexual maturity in their second year, and most individuals likely do not survive to year three. This growth model suggests that most pearl darters spawn just one spring and will not reach a second year of spawning. Compared to other *Percina* species, this is relatively common. For instance, *Percina vigil* typically does not live to year three and reaches sexual maturity at year one (Heins & Baker, 1989). *Percina stictogaster* (Eisenhour *et al.,* 2013), and *Percina suttkusi* also only live to a maximum of three years (Thompson, 1997). However, *Percina sciera* can live upwards of five years, and *Percina lenticula* is thought to live even longer (Page and Smith, 1970).

There is also a clear size difference amongst the pearl darters in the varying rivers, with a general pattern of larger adults being found in smaller systems. The modeled

maximum size in the Leaf and Chickasawhay Rivers was larger than that in the Pascagoula River. While not presented here (insufficient sample size), adults in smaller tributaries (e.g. Black Creek, Okatoma Creek, Bouie River) are substantially larger than adults in the Pascagoula River. It is not clear if these fish may be living longer, acquiring more resources for faster growth, or possibly allocating more resources to growth. This allocation to growth could be delaying reproduction ,which could lead one to infer that the species is spawning earlier in different tributaries. This refers to the THLM where the stability of the environment can affect the behaviors of the species. Further sampling and comparison to other *Percina* species will need to be conducted to better understand the pearl darter life history.

Future Direction

The 2021 sampling efforts, though relatively effective in catching pearl darters, did have some limitations. For instance, working in a larger river system means that heavy rain can result in dangerous conditions for sampling as the water level is high. The 2021 summer had the most rainfall recorded in over 50 years, which resulted in minimal sampling capabilities. There was also a cold snap in February of 2021 that could have interrupted the usual allocation of resources. Additionally, the sex ratio found on the sampled sand bars hints that there are other pearl darters residing elsewhere. These three factors limited the 2021 sampling efforts and were the impetus for the initiation of further sampling in 2022.

The 2022 sampling, that started in January 2022, will be repeating the sampling work in 2021 with the inclusion of additional sites. The hope is to find where the females are residing prior to moving into the sampled spawning habitat. This could help in

explaining the sex ratio seen in the 2021 sampling efforts. In addition to the seining conducted in 2021, trawling of the Chickasawhay, Leaf, and Bouie rivers will be conducted to collect individuals in the deeper habitat that may not have been reached with seining. The trawling also allows for collection of more *Percina nigrofasciata* and *Percina suttkusi.* So far, the trawling has allowed for great numbers of *Percina suttkusi* and *Percina nigrofasciata* specimen collections. The next step for the already collected pearl darter samples will be measuring and determining stages of the oocytes to better contextualize the timing of and investment (GSI) in reproduction. The measuring of the stages will allow the determination of if individuals are spawning more than once in a season. While not presented here (results pending), this work has been conducted alongside larval sampling, which is also being continued into 2022 to identify the larval fish genetically. This sampling will help in documenting whether the eggs are hatching and the species is reproducing effectively. Only knowing GSI cannot tell whether the larvae are successful in survival as environmental factors can affect the hatching. For instance, eggs on substrate near poor land use could result in the eggs being buried in the sediment, making the larvae unable to hatch (Stearman *et al.,* 2014). Our goal is to ultimately understand spatial and temporal aspects of larval recruitment to inform conservation efforts and to confirm whether the population of pearl darters is reproducing sufficiently.

For further study, along with starting sampling earlier in the year, the next step will be to analyze the number, size, and developmental stage of the eggs for the species observed. This analysis could provide further insight into how many clutches each species may have in a spawning season. It is common knowledge that *Percina vigil* has

multiple clutches in a spawning season, which was found by analyzing the fecundity of the species (Heins & Baker, 1989). Though not yet known for the pearl darter, considering the relatedness of the two species, the pearl darter could potentially be producing multiple clutches in a season as well.

Additional research on the pealr darter could be conducted using some past studies done on other Darters (Percidae) as references. Firstly, Eisenhour *et al.* (2013) conducted a study on the frecklebelly darter, *Percina stictogaster*, in the Red River, Kentucky, similarly to the future efforts of pearl darter research. Though in a different river system, the frecklebelly darter shares some similarities with the pearl darter in terms of habitat preference. This leads to consideration that some of the behaviors observed in the frecklebelly darter may also be present in the pearl darter. For instance, the freckelbelly darter adults occupy faster moving water than the juveniles (Eisenhour *et al.,* 2013). Considering there appears to be some habitat preference for spawning for the pearl darter, there may exist habitat preference based on life stage as well.

Bennett *et al.* (2016) discussed the Trilateral Life History Model (TLHM) of three different species, the red shiner (*Cyprinella lutensis*), the bluntnose minnow (*Pimephales notatus*), and the orangethroat darter (*Etheostoma spectabile*) in the Midwestern US. Orangethroat darters were found to have variation in egg size related to the stability of current water velocities (Bennet *et al*., 2016). Considering that the pearl darter VBGM showed that there were larger individuals in the smaller river system, there is a possibility that a similar pattern may be present in the eggs of the pearl darter. Overall, the TLHM may be useful in understanding the evolution of life history in the pearl darter, and variability among the three rivers in which they are found (Bennett *et al.,* 2016).

Stearman *et al.* (2014) conducted a life history study on the redfin darter, *Etheostoma whipplei*, in Cypress Creek, Arkansas. This darter species is adaptable in its habitat occupation but is found mostly in headwater streams residing on gravel substrate. This study consisted of a 12-month long survey in which sampling was conducted along with observations made using a snorkeling survey (Stearman *et al.,* 2014). Although the Pearl River drainage waters are not clear enough to conduct a snorkeling survey, the other sampling techniques used in assessing reproduction in the Stearman *et al.* study are similar to those that will be used in future pearl darter research. A major takeaway from this study is that the year long sampling and dissection of the species provided a much more definitive GSI curve than that seen in this 2021 pearl darter survey. A 12-month sampling of the pearl darter could also aid in determining habitat preference changes in and out of spawning period.

Lastly, the study conducted by Ruble *et al.* (2019) analyzed the reproductive efforts of captive yazoo darter, *Etheostoma raneyi*. Although the yazoo darter is in a different genus than the pearl darter, they both are in the Percidae family and are closely related. The study conducted on the yazoo darter included the collection of reproductive adults that were then placed into breeding groups during peak spawning months (Ruble *et al.,* 2019). Although this may be difficult to do with the federally threatened pearl darter, attempting to captively propagate the pearl darter could offer a clue into their reproductive behavior. It could potentially confirm what the sex ratio observed in 2021 hints at, which is that females remain in a separate habitat until they are ready to spawn, upon which they meet the males at the known spawning habitat.

APPENDIX A: IACUC APROVAL LETTER

THE UNIVERSITY OF
SOUTHERN MISSISSIPPI

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

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

NOTICE OF COMMITTEE ACTION

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

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

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

Samuel Bruton, PhD
Director, Office of Research Integrity

March 3, 2021
Date

APPENDIX B: COLLECTIONS PERMIT

D. Acceptance of this permit serves as evidence that the permittee understands and agrees to abide by the terms of this permit and all sections of title 50 Code of Federal Regulations, Parts 13 and 17, pertinent to issued permits. Section 11 of the Endangered Species Act of 1973, as amended, provides for civil and criminal penalties for failure to comply with the permit conditions. In addition, the permittee shall have all other applicable Federal, Tribal, State, and/or local government permits prior to the commencement of activities authorized in this permit.

E. Permittee is authorized to take (capture, handle, and euthanize) the federally threatened pearl darter (*Percina aurora*) for a 2-year study on life history, as specified in permittee's August 14, 2020, application and supplemental information submitted on December 2, 2020, and as conditioned below:

1. Permittee will target reproductive darters 1-2 years of age and drifting larvae estimated to be <14 days of age.

2. Pearl darters may be captured via seines and larval drift nets throughout the presumed spawning season (March - May).

3. Up to 5 reproductive adults per sampling site, for a total of up to 30 adults over a 2-year period, may be retained and euthanized for dissection to document reproductive investment through gonadal and somatic measurements.

4. Up to 100 larvae captured in drift nets over a 2-year period may be retained and preserved in ethanol for genetic analysis.

5. If collected fishes must be held temporarily during survey efforts, they must be kept in containers with flowing water (i.e., aerated holding buckets, submerged seine). If an aerated bucket is used, the bucket shall be kept cool (out of direct sun) and clean (e.g., did not previously contain formalin or other preservatives or toxins) and shall not contain other species (e.g., crayfish or other fish species). Holding shall be limited to 30 minutes.

6. Captured fish shall be released as close as possible to the point of capture. They shall be released by hand at the substrate level to avoid higher risk of predation and allowed to swim under their own power from the hand to the substrate.

7. If a federally listed fish species that is not included in this permit is found in the action area or outside of its currently known range, the permittee shall promptly notify the appropriate USFWS Field Office (https://www.fws.gov/ecological-services/map/directory.html#AL).

8. All dead larval and adult darters must be preserved according to standard museum practices and properly labeled with collection dates and site identification, including latitude, longitude, river-mile if appropriate, and site condition. At the conclusion of the study, the preserved specimens must be deposited at the Mississippi Museum of Natural Science, 21489 Riverside Drive, Jackson, Mississippi, unless otherwise authorized by the USFWS' species recovery lead (<https://www.fws.gov/southeast/pdf/data/recovery-leads.pdf>).

F. No unintentional/accidental injury or mortality is expected to occur to federally listed species covered under this permit. In the event that any accidental injury or mortality occurs, all activities must cease and the injury or mortality reported immediately (not to exceed 1 business day) to the appropriate USFWS species recovery lead (<https://www.fws.gov/southeast/pdf/data/recovery-leads.pdf>) and Southeast Region Recovery Permit Coordinator at the address and telephone number noted in Condition L., below. Based upon consultation between these offices, a decision will be made as to whether any of the authorized activities can continue. Decisions will also be made

concerning the disposition of any dead or injured specimens. The permittee shall provide a written statement to the USFWS species recovery lead(s) and Southeast Region Recovery Permit Coordinator, which documents the cause of the injury/mortality, and identifies the remedial measures employed by the permittee to eliminate future mortality/injury events. The final decision on remedial measures and disposition of specimens rests with the USFWS.

G. This permit is non-transferable.

H. Permittee must carry a copy of this permit at all times when conducting the authorized activities. Shipments of collected biological materials should also be accompanied by a copy of this permit. Note that this permit is limited to the above activities and identified species.

I. Issuance of this permit does not constitute permission to conduct these activities on national wildlife refuges or any other public or private lands; such permission must be obtained separately from the appropriate landowner or land manager before beginning these authorized activities. This permit, neither directly nor by implication, grants right of trespass.

J. Upon locating a dead, injured, or sick federally listed species, under circumstances not addressed in this authorization, initial notification must be made immediately to the USFWS Field Office in the State in which the specimen is found (https://www.fws.gov/ecological-services/map/directory.html#AL). Notification should also be made by the next work day to the USFWS' Southeast Region Permit Coordinator identified in Condition L., below. Those offices will confer with the USFWS' Division of Law Enforcement as appropriate and determine next steps. Care should be taken in handling sick, injured, or dead specimens to ensure effective treatment or to preserve biological materials for later analysis. In conjunction with the care of sick or injured endangered or threatened species, and the preservation of biological materials from a dead individual, the finder should take responsible steps to ensure that the site is not unnecessarily disturbed.

K. An annual report summarizing the authorized activities must be submitted to the USFWS' Regional Office(s) identified in Condition L., as well as to the appropriate species recovery lead (<https://www.fws.gov/southeast/pdf/data/recovery-leads.pdf>) and the USFWS Field Office in the State where activities occurred (https://www.fws.gov/ecological-services/map/directory.html#AL) by January 31 following each year that this permit is in effect. When possible, electronic copies shall be submitted in lieu of hard copies in MS Word, Portable Document Format, Rich Text Format, or other file format that is compatible with the receiving office. Each report should include, at a minimum, the following information:

For All Species:

1. The name(s) and organization affiliation of all members of the survey crew.

2. The date(s) of the survey(s).

3. Locations of the survey sites. Locations shall be noted using figures, maps, and by referencing a common coordinate system (e.g., latitude longitude, universal transverse mercator system, etc.).

4. Survey methods used and a map showing the survey location, with state and county designations, along with a description of the area sampled, noting biotic and abiotic features that might influence sample composition (e.g., water quality data, including velocity, visibility, temperature, DO, pH, turbidity, and conductivity). The length of

each survey reach and the latitude/longitude of the start and end location of each stream reach should also be included.

5. Species abundance and richness at each sample event, including parameters describing sampling effort.

6. The results of the sampling, with discussions and interpretations of the data in context to recovery of the species.

7. A description of any mortality, injuries, deformities, or other abnormalities observed and disposition of specimens.

8. Copies of all published data and reports.

9. Disposition of all preserved specimens.

IF NO ACTIVITIES OCCURRED OVER THE COURSE OF THE YEAR, INDICATION OF SUCH SHALL BE SUBMITTED AS AN ANNUAL REPORT.

L. For purposes of monitoring compliance and administration of the terms and conditions of this permit, the contact office of the U.S. Fish and Wildlife Service is:

U.S. Fish and Wildlife Service (Southeast Region)
Attn: Recovery Permit Coordinator (Ecological Services)
1875 Century Boulevard
Atlanta, Georgia 30345-3301
Telephone: 404/679-7097
Facsimile: 404/679-7081
PermitsR4es@fws.gov

END

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