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## Aspects of the Reproductive Biology and Growth of the Mississippi Silvery Minnow, *Hybognathus nuchalis* (Agassiz, 1855) (Teleostei: Cyprinidae) from the Pearl River, Louisiana.

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# Aspects of the Reproductive Biology and Growth of the Mississippi Silvery Minnow, *Hybognathus nuchalis* (Agassiz, 1855) (Teleostei: Cyprinidae) from the Pearl River, Louisiana.

## Abstract

The reproductive biology and growth of the Mississippi Silvery Minnow, *Hybognathus nuchalis*, is described from multiple sites in the Pearl River, Louisiana. Individuals were collected from August 2011 to August 2012. Ovarian weights, expressed as a percentage of body weights, peaked in December. Size structure ranged from 29.0 to 60.0 mm SL for females and 25.0 to 56.0 mm SL for males. Mature ova were found from November to January. Females reached first maturity (L50) at 37.0 mm SL and L50 for males is at 41.0 mm SL. Sex ratio (females:males) is biased towards females ( $\chi^2 = 18.57$   $p < 0.05$ ). Fecundity of mature individuals ranged from 118 to 830 ova (mean  $433 \pm 256.8$  SD) in fish 30-50 mm SL. There was negative allometric growth for both sexes and there was a significant relationship significant between SL and body weight for both sex ( $R^2 = 0.9$ ,  $p < 0.05$ ).

## Keywords

Cyprinidae, life-history, reproduction

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## Cover Page Footnote

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## INTRODUCTION

The number of basic life-history studies have declined over the past several decades as new, technologically advanced approaches (i.e. DNA sequencing) have taken over the biological landscape (Noss 1996, Arnold 2003, Greene 2005, Tewksbury et al. 2014). Despite reductions in the numbers of autecological studies, there still remains a need to gather basic information on ecology, diet, habitat, and reproductive biology for many species, including many of the most abundant and widely distributed species. Fortunately, natural history museums contain a plethora of samples that can provide relevant life-history data, which, in turn, can be used as a foundation for the development of conservation and management plans (Ponder et al. 2001, Suarez and Tsutsui 2004).

The Southeastern United States possesses the most diverse temperate ichthyofauna in the world. One of the more speciose river systems in the region is the Pearl River, which contains >200 fish species (Ross, 2001). The Pearl River has been identified as a high priority focus for conservation attention within the eastern Gulf Coastal Plain and northern Gulf of Mexico ecoregions. Approximately fifty percent of the Pearl River basin is forested, with logging and timber processing primarily occurring in the lower half of the basin. Fortunately, from a fish community perspective, the Pearl River is one of the most well-studied river systems in the southeast, as substantial collections of fish specimens consistently have been archived at museums in the region since the 1950s (Piller et al. 2004, Geheber and Piller 2012, Piller and Geheber 2015).

In August 2011, the Pearl River was subjected to a weak black liquor spill (between 25 and 75 million gallons) from a paper mill causing a reduction of dissolved oxygen in the river. This resulted in substantial, short-term loss of fish in a large stretch of the main channel. A detailed post-spill survey of the fish community was undertaken and resulted in the sampling of large numbers of fishes from both the impacted area and upstream areas that were permanently archived in a regional natural history collection. Piller and Geheber (2015) quantified resilience and recovery of fish assemblage structure in the impacted area following the event. This study demonstrated rapid recovery in an aquatic system, and also further demonstrated the value of continuous, long-term, data collections, which enhance our understanding of fish assemblage dynamics. A large number of specimens were sampled during this period and form the basis of this study.

The Mississippi Silvery Minnow (Cyprinidae: *Hybognathus nuchalis*) is widespread in the middle and lower Mississippi River basin, as well as throughout river systems along the Gulf Coast including the Mobile, Pascagoula, and Pearl

River basins (Page and Burr 2011) (Fig. 1A). It is commonly found in small to moderate-sized streams in areas of moderate current over silt and sand substrates. Prior to the Pearl River fish kill in 2011, it was an abundant species in the Pearl River basin (Geheber and Piller 2012). The principal diet of the species consists of “ooze”, a mixture of algae and diatoms (Flemer and Woolcoot 1966, Whitaker 1977).

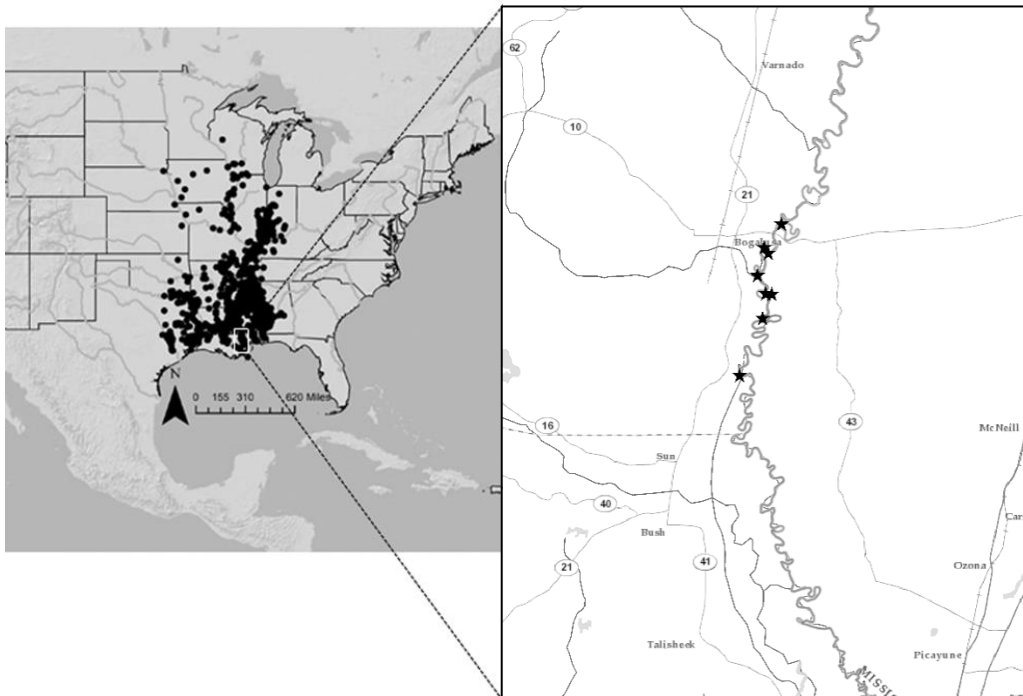


Figure 1. A) Distribution of *Hybognathus nuchalis* based on vouchered museum records (www.fishnet2.net, August 2018), and B) distribution of sampling locations (stars) in the Pearl River, 2011-2012.

Despite knowledge of the distribution, habitat, and feeding habits of the Mississippi Silvery Minnow, little information about its reproductive biology is known, other than brief comments on the timing of spawning in other geographic areas (Forbes and Richardson 1920, Becker 1983). The purpose of this study is to characterize aspects of the reproductive biology of the Mississippi Silvery Minnow from the Pearl River, Louisiana, using archived museum specimens and to compare the results to congeners. Understanding the reproductive life history of a species is important when trying to understand changes in abundance, because the persistence

of any species is measured by its ability to successfully produce offspring (Wootton 1990; Moyle and Cech 2004).

## **MATERIALS & METHODS**

### **Field-Sites Description**

The Pearl River is a large Gulf Coastal Plain system that originates in east-central Mississippi and flows in a southwesterly direction along the eastern edge of the Louisiana border towards its mouth at Mississippi Sound in the Gulf of Mexico (Ross, 2001). Detailed information regarding the Pearl River study area is provided in Geheber and Piller (2012).

### **Fish Sampling and Reproductive Evaluation**

Fish sampling followed the protocols provided in Piller and Geheber (2015) (Fig. 1B). Fishes were sampled from all sites from the eight historic sampling sites of the lower Pearl River survey initiated by the late Royal Suttikus, Tulane University (Geheber and Piller 2012), using a 10 x 6ft seine (3/16" ace mesh). Specimens were fixed in 10% commercial grade formalin, and later transferred to 70% ethanol. Specimens examined for this study were deposited in the Southeastern Louisiana University Vertebrate Collection (SLU) (Appendix 1). Specimens originally were sampled from the main channel of the Pearl River during the months of August, September, October, November, and December 2011, as well as January, April, May, June, July, and August 2012. Specimens from February and March 2012 were not available for examination. Specimens included in this study were sampled from localities above and below the area impacted by the black liquor spill.

Total wet weight was determined to the nearest 0.1 g and standard lengths (SL) measured to the nearest millimeter for studied fish. Ovaries were removed intact, blotted to remove excess moisture, and weighed to the nearest 0.0001g. Clutch size was used to estimate fecundity (F) (Heins and Rabito 1986). Oocytes were separated and counted in a petri dish containing water from gonads in stages MA and MR. The gonadosomatic index (GSI) was calculated as the ratio of ovarian weight to total body weight. Stages of ovarian condition were evaluated following the criteria of Heins and Rabito (1986) and Heins et al. (1992) and are described in Table 1. Analysis of sex ratios followed the criteria of Sparre and Venema (1997). The statistical significance of the results was established by a fit to the Chi-squared test ( $\chi^2$ ), using  $\alpha = 0.05$  to assess significance. Condition factor was assessed with Fulton's condition factor (K) (Froese, 2006). Model growth was evaluated by linear regression, calculating the  $a$  and  $b$  values of the equation  $W = aL^b$ , where  $W =$  is

body weight,  $L$  is standard length,  $b$  is the growth exponent or length-weight factor, and  $a$  is a constant. The  $a$  and  $b$  values were estimated using a linearized form (Froese, 2006). Population structure was analyzed by grouping the data into SL ranges following the criteria of Sturges (1926). A variance analysis allowed the identification of significant differences by size and two-way analysis of variance to assess differences between sexes. The Tukey-Kramer post-hoc test was used to identify significant differences ( $p < 0.05$ ). Size at first maturity ( $L_{50}$ ) was related to the standard length using the logistic regression model to fit sigmoid curves according to the following equation:  $M(L) = 1 / (1 + e^{-(aL+b)})$ . Confidence limits were derived by Bayesian inference based on stochastic simulation.

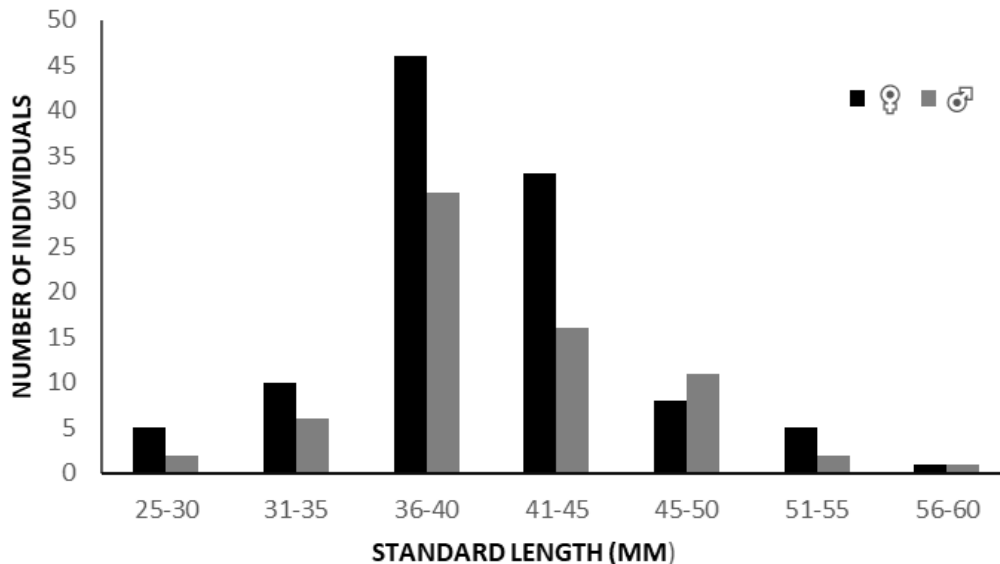
**Table 1.** Stage ovarian condition describing by the criteria of Heins and Rabito (1986) and Heins et al. (1992).

Stage	Description of ovaries
Latent (LA)	Ovaries very small, thin, and transparent to slightly translucent. Maturing oocytes, if present, yolkless or vitellogenic with nucleus visible.
Early maturing (EM)	Ovaries small to moderate in size and translucent to white (opaque). Maturing oocytes small to moderate size and translucent to white with nuclei obscured by yolk.
Late maturing (LM)	Ovaries small to greatly enlarged and white to cream. Maturing oocytes moderate to large size and white to cream.
Mature (MA)	Ovaries moderate sized to greatly enlarged and cream to yellow. Two separate groups of follicular oocytes, which are opaque (usually) and cream to yellow without chorions separate from the yolk.
Ripening (MR)	Ovaries moderate sized to greatly enlarged and cream to yellow. Two distinct groups of follicular oocytes. The larger, ripening oocytes are translucent (most often) to transparent with chorions separated from the yolk.
Ripe (RE)	Ovaries moderate sized to greatly enlarged and cream to yellow. One group of maturing follicular oocytes, which are moderate to large sized and white to cream. Ripe ova concentrated in the lumen of the ovary (usually posterior) and translucent to

transparent (usually) with chorions separated from the yolk.

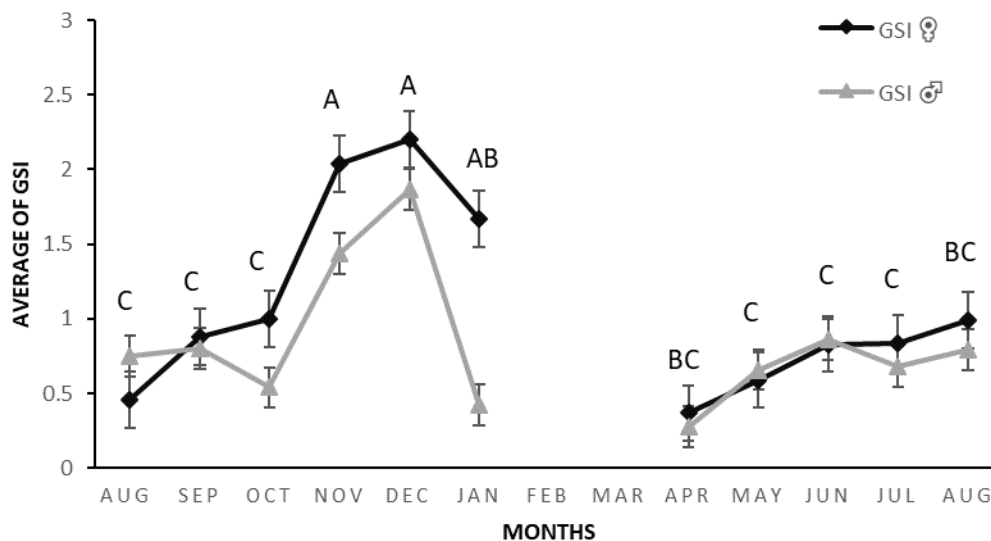
## RESULTS

Two-hundred twenty individuals from the Pearl River were examined. Dissections included 109 females, 70 males, and 41 indeterminate individuals. Standard length frequency histograms indicate a range of 29.0 mm to 50.0 mm SL for females, with a mean size  $37.0 \pm 5.79$  mm SL. The size range of males is 25.0 mm to 56.0 mm SL (Fig. 2) with a mean size of  $39.0 \pm 5.90$  SD mm SL. There were significant differences between sizes among sampling months for both sexes,  $F_{10,98} = 8.17$ ,  $p < 0.0001$  for females, and  $F_{10,59} = 13.30$ ,  $p < 0.0001$  for males. November (mean 44.0 mm SL females and 45.0 mm SL for males), December (45.0 mm SL for females and 44.0 mm SL for males) and August 2012 (41.0 mm SL for females and 42.0 mm SL) showed the largest sized individuals across all months. The smallest fishes were from April for females (26.0 mm SL), and May for males (25.0 mm SL). Sex ratio (females:males) is female biased, and a chi-squared test revealed significant heterogeneity ( $X^2 = 18.57$ ,  $p < 0.05$ ), however, in November 2011 and August 2012 the sex ratio was male biased (0.5:1).



**Figure 2.** Standard length-frequency histogram for females (♀) and males (♂) of all the individuals from all months of *Hybognathus nuchalis* from the Pearl River, Louisiana.

In April and May, the highest number of indeterminate individuals was sampled (stages of ovarian maturation LA, EM, ML, Table 1). Reproductive activity increased markedly from November to January, and then sharply declined until a minimum value was reached in April 2012 (Fig. 3). Mean GSI peaked in December for both sexes. There was no significant difference among GSI values for males ( $F_{10,59} = 0.88$   $p > 0.05$ ) across the seasons, however there was a significant difference in GSI values for females ( $F_{10,98} = 15.54$   $p < 0.05$ ). A Tukey-Kramer test showed November (mean 2.0), December (mean 2.5), and January (mean 1.7) with the highest GSI values (Fig. 3). K values were constant through the year and were not significantly different ( $F_{10,58} = 13.03$ ,  $p > 0.05$ ). Mature ovaries in stages MA, MR



**Figure 3.** Monthly variation in the gonadosomatic index (GSI) for females (♀) and males (♂) of *Hybognathus nuchalis* from August 2011 to August 2012. No data were available for February and March. Letters (A, B and C) indicate significant differences (Tukey-Kramer test) in GSI values between months for females ( $F_{10,98} = 15.54$   $p < 0.05$ ). No statistical difference for males ( $F_{10,59} = 0.88$   $p > 0.05$ ).

showed negative allometric growth for both sexes. There was a significant relationship between SL and body weight for both sexes ( $R^2 = 0.9$ ,  $p < 0.05$ ).



## DISCUSSION

The reproductive biology of the Mississippi Silvery Minnow was investigated from the Pearl River, Louisiana and represents the first comprehensive study of the reproductive biology for this species. The reproductive variables evaluated in this study indicate that *H. nuchalis* has a spawning season from November to January. This is substantially earlier than what has been reported from populations in more northern latitudes (Forbes and Richardson 1920, Becker 1983). In Wisconsin, Becker (1983) noted that *H. nuchalis* spawns from April through June. In Illinois, Forbes and Richardson (1920) noted the collection of reproductively mature individuals in June. Clearly, differences in the timing and duration of spawning among widespread cyprinids, such as *H. nuchalis*, can be explained by differences in latitude and associated water temperatures (Wilde et al. 1999; Cross 1967; Lehtinen and Layzer 1988; Farringer et al. 1979).

Leuciscids (*sensu* Tan and Armbruster 2018) exhibit different methods of spawning that seem to be dependent on temperature, day length, precipitation, and flow conditions (De Vlaming 1974). Gonadosomatic indices revealed that the spawning season of *H. nuchalis* is not prolonged. Beginning in November, female *H. nuchalis* begin to divert energy toward reproduction resulting in increases in adult mean GSI. This is followed by decreases in GSI in the population, which are indicative that a spawning event occurred. The first occurrence of a decrease in GSI was in April and this continues until August. The presence of ovaries at mature stages of development (MA, Mature; MR, Ripening; RE, Ripe) in November and December, and recruitment of mature oocytes during this period confirms a single reproductive period for *H. nuchalis*. Other species of *Hybognathus* (*H. hankinsoni* and *H. placitus*) also show a single reproductive peak (Copes 1975, Lehtinen and Layzer 1988). This differs from other North American leuciscid species, which are known to be multiple spawners and show two reproductive peaks (Wallace and Selman 1981; Heins and Rabito 1986; Wilde et al. 1999).

Reproductively mature females of *H. nuchalis* possessed two groups of vitellogenic ova in their ovaries including smaller, maturing ova and larger, mature ova. This phenomenon is not unique to *H. nuchalis* as it has been demonstrated for other species of cyprinids (Heins and Rabito 1986). *Hybognathus nuchalis* produced multiple clutches, and its fertility is lower in comparison to other species of *Hybognathus*, such as *H. placitus*, which possessed 417 to 4,134 mature ova (Taylor and Miller 1990).

The size class distribution observed in this study suggests that *H. nuchalis* is a relatively long-lived species with good annual survival in the Pearl River. The presence of multiple individuals at several size classes also suggests that *H. nuchalis* is iteroparous. The maximum size reported for *H. nuchalis* varies geographically. Becker (1983) reported a maximum size of 107 mm SL from Wisconsin, 123 mm SL from Ohio (Trautman, 1981), whereas Ross (2001) noted a maximum size of 152 mm TL from Mississippi, which is substantially larger than the individuals analyzed in this study from the Pearl River (<60 mm SL). Other species of *Hybognathus* show maximum sizes of around 100 mm SL (*H. hankinsoni*, Becker, 1983; *H. placitus* Lehtinen and Layzer 1988; *H. hayi* Ross, 2001; *H. regius* Raney 1939).

The early life history of many cyprinids includes a drifting egg and larval stages, and the negative impacts of river modifications, particularly on flow and substrate, have been well-documented (Stanford and Ward, 1979; Cross et al., 1985; Luttrell et al., 1999). Other species of *Hybognathus* from other regions (i.e. *H. placitus* and *H. amarus*) are known to produce semi-buoyant non-adhesive eggs that develop and hatch as they float downstream (Sliger 1967, Platania and Altenbach 1988). *Hybognathus regis* also produces non-adhesive eggs, but the eggs are attached to vegetation instead of being semi-buoyant (Etnier and Starnes 1993). For *H. nuchalis*, it is unknown whether semi-buoyant eggs are produced. Vegetation in the main channel of the Pearl River is very limited, so it is assumed that *H. nuchalis* also produces semi-buoyant drifting eggs, although no study has assessed this aspect of its life-history.

A variety of factors have contributed to the loss of biodiversity in the Pearl River including reservoir and navigation canal construction, which have resulted in changes in the geomorphology of the Pearl River and directly impacted many benthic species (Piller et al. 2004, Tipton et al. 2004). In addition, there is substantial variation in discharge levels in the Pearl River (Geheber and Piller 2012), and this also is due, in part, to the Ross Barnett reservoir in the upper portion of the basin. This is relevant since the reproductive biology of some minnows is directly dependent on water availability and it is essential that sufficient flow is maintained during the appropriate time periods to allow for egg/larval drift, and adult and sub-adult migration. Periods of increased discharge are needed during the spawning season for successful synchronized spawning events for many species. For example, *Hybognathus amarus* (Rio Grande Rive), a close relative of *H. nuchalis*, was found to show very poor recruitment when spring river pulses were low or non-existent (Turner et al. 2010). Fortunately, flow levels in the Pearl River are often very high during the time-period in which *H. nuchalis* spawns. However, in some years winter rainfall can be low, and this is when appropriate discharge

levels from the reservoir must be maintained for successful synchronized spawning and the movement of early life-history stages of *H. nuchalis* are not impacted.

Despite its widespread distribution and abundance throughout its range, little information on the basic reproductive biology and growth of *H. nuchalis* was known prior to this study. This study provides new information for an understudied species and provides data on fecundity, GSI, growth, the timing of reproduction. This new information revealed some differences in the timing of reproduction relative to other populations of *H. nuchalis* from more northern latitudes. The information gathered in this study adds relevant data on the reproductive biology and growth of a southeastern cyprinid and further emphasizes the importance of regional life-history studies, particularly for widespread species. Basic life-history studies are needed for many other fishes in the region to allow resource managers to appropriately manage species and systems based on sound natural history information.

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## APPENDIX 1

### Specimens Examined

SLU numbers correspond to the Southeastern Louisiana Vertebrate Museum, Ichthyology Collection. Pearl River, SLU 7101 (August 2011), SLU 7165 (September 2011), SLU 7286 (October 2011), SLU7329 (November 2011), SLU 7501 (December 2011), SLU 7517 (January 2012), SLU 7772 (April 2012), SLU 7912 (May 2012), SLU 7981 (June 2012), SLU 8077 (July 2012), SLU 8178 (August 2012).