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Reproductive timing of the Scarlet Shiner (*Lythrurus fasciolaris*) in Northern Alabama

Abstract

The Scarlet Shiner (*Lythrurus fasciolaris*) is a cyprinid species widely distributed in parts of the Ohio, Cumberland and Tennessee river drainages of the United States. The objective of this study was to determine the Scarlet Shiner's reproductive schedule. Maturation of ovaries and oocytes was determined through the categorization of developmental stages from early maturing to ripe, along with the calculation of monthly gonadosomatic index (GSI) measurements over two reproductive seasons in 2012 and 2015. In both years reproductive competence began in April. Average monthly GSI for females peaked in May, followed by a slow decline through August. Average clutch size was largest both years in April and May, although individual females were found with late developmental stage oocytes as late as August. Compared to other studied sympatric cyprinid species, the Scarlet Shiner was found to be a relatively late spawner with an extended spawning tail well into the summer.

Keywords

cyprinid, stream fish, oocyte, GSI, clutch size, spawning

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Cover Page Footnote

J. Mann, K. Hodgskins, and S. Greenleaf helped with the fieldwork on this project. K. McDerby helped with much of the oocyte imaging and counting. K. Hodgskins created the maps in Figure 1. The comments of two reviewers improved the manuscript. Co-author T. Taylor was supported on this project in partial fulfillment of the requirements for a Master of Science degree by a stipend from the Louis Stokes Alliance for Minority Progress at the University of Alabama in Huntsville. Material support for this project came from in part from a USDA grant, 1890 Institution Capacity Building Grants Teaching Program, #2012-02422, to B. Stallsmith.

INTRODUCTION

The southeastern United States supports a rich diversity of freshwater fish species. Life history details such as reproductive schedule for many of these species are poorly known, even for some of the more widely distributed species. One such species is the Scarlet Shiner (*Lythrurus fasciolaris*), a small cyprinid found throughout portions of the Ohio, Cumberland, and Tennessee river drainages in upland areas. The Scarlet Shiner inhabits pools and glides of small to medium, clear, rocky streams of moderate gradient (Hopkins and Eisenhour, 2008).

Scarlet Shiner spawning activity has been observed to be associated with nest-building fish species such as members of the genera *Lepomis* and *Nocomis* from mid-May to late August (Hopkins and Eisenhour, 2008). In Alabama its spawning is often over the nests of nest-building species such as River Chub (*Nocomis micropogon*) and Longear Sunfish (*Lepomis megalotis*) (B. Stallsmith, personal observation). When in spawning condition the species is strongly sexually dimorphic, with the bright coloration and aggressive behavior of dominant males induced by high levels of the potent androgen 11-ketotestosterone (Schade and Stallsmith, 2012). Reproductive adults are 41–90 mm in length, and life expectancy is probably 3 years (Ross, 2001). Adults feed on insects taken in mid-water and at the surface, as well as midge larvae and mayfly nymphs in the substrate (Boschung and Mayden, 2004).

The objective of this study was to determine the reproductive schedule, *sensu* Heins and Rabito, 1986, of the Scarlet Shiner. This involved examining the maturation of oocytes and ovaries by developmental stages, as well as calculating monthly gonadosomatic index (GSI) for females and males over a total of two years in 2012–2013 and 2014–2015. The number of oocytes in different developmental stages was measured during the observed reproductive season which enabled the calculation of clutch size, the number of larger, vitellogenic oocytes often termed mature oocytes available for spawning in the near future (Heins and Baker, 1993).

MATERIAL & METHODS

Collection Sites and Fish Capture

Collections of Scarlet Shiners were made monthly from a 10 km stretch of the Flint River in Madison County in northern Alabama from April, 2012, to June, 2013, and September, 2014, to August 2015. The Flint River originates in southeastern Lincoln County, Tennessee and flows south through Madison County, Alabama into the Tennessee River southeast of Huntsville, Alabama. The river drains 141,640 hectares of Madison County, Alabama and Lincoln County,

Tennessee (Abdi et. al., 2009), and its major branches total 562 km. The main stem of the river is free-flowing along its 111 km length. Within the sampling area, clear to moderately turbid waters flow over substrates of exposed Tusculumbia limestone and Fort Payne chert. alluvial deposits of boulders, large cobble, small cobble, sand, silt, and mixtures of each are present and create an alternating succession of runs, riffles, and pools (Hodgskins et al., 2016). Collections were made at three sites: Oscar Patterson Road (34° 52' 50" N, 86° 28' 50" W), Mt. Carmel (34° 48' 20" N, 86° 28' 20" W), and Three Rivers (34° 49' 22" N, 86° 28' 59" W) (Figure 1). No effort was made to collect individuals of a specific size or sex, but the aim was to collect 30–40 adults each month. The collection sites were typical of Scarlet Shiner habitat; medium-sized, clear-water streams with rock, pebble, and gravel substrates and medium water flow (Boschung and Mayden, 2004). Water temperature was also recorded during each collection using an alcohol thermometer.

Kick-seine and cast-net techniques were used for capturing Scarlet Shiners. The 3-mm mesh seine net was 3.5 m long, and 1.2 m deep. The cast net used contained 0.75 mm mesh with a diameter of 2.3 m. After capture, specimens were euthanized on site using 2 ml of a clove oil solution (90% EtOH:10% clove oil) added to ~400 ml of river water in a plastic jar, and then fixed in 10% phosphate buffered formalin.

Data Collection

Standard length (SL) and mass were recorded for each preserved specimen. SL was measured using a digital caliper (Fisher Scientific) and recorded to the nearest tenth of a millimeter. Each specimen was blotted to remove excess fluid before being weighed on an Ohaus® Explorer balance to the nearest 0.001 g. The mass of excised gonads was also recorded to the nearest thousandth of a gram.

An adult was classified as a specimen > 41 mm in SL. This decision was based on observation of specimens less than 41 mm SL having little visible gonadal tissue. Gonadosomatic Index (GSI) was determined for each adult. This was calculated as a percentage using the somatic mass (total mass minus gonadal mass) and gonadal mass in the following formula: $GSI = (\text{gonadal mass} / \text{somatic mass}) \times 100$ (Jolly and Powers, 2008; Stallsmith et al., 2015; Hodgskins et al., 2016; Thompson et al., 2017).

Reproductive evaluation

Images of ovaries and oocytes were captured using an Olympus SZX7 dissecting microscope with an Olympus DP72 camera. The images were later analyzed for aspects of ovarian and oocyte maturation using the Olympus cellSens Standard imaging software (Ver. 1.5) included with this camera. Each image was

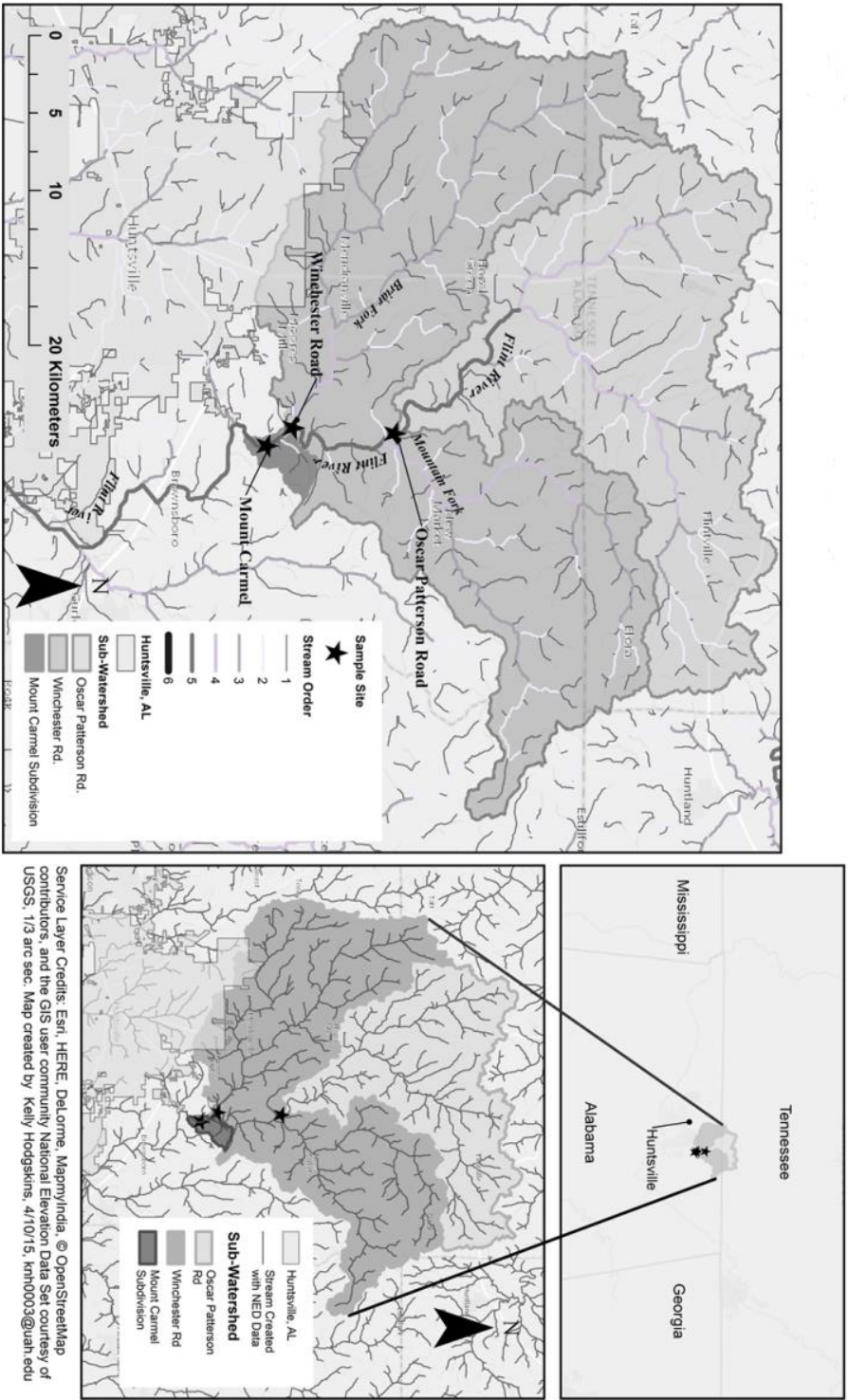


Figure 1. Map of fish collecting sites at the Flint River in Madison County, Alabama.

captured at 8.4X (1.6X x 4.0X) magnification and saved as a .tiff file to ensure long-term integrity of the digital information.

The method developed by Núñez and Duponchelle (2009) was used to assess ovarian maturation. Ovaries were divided into five stages of maturation. Latent (stage I) ovaries are usually opaque, small in diameter, and contain latent oocytes only. Early maturing (stage II) ovaries inhabit a larger portion of the abdominal cavity and contain white and cream colored oocytes varying in size. Late maturing (stage III) ovaries are loaded with yellow to orange vitellogenic oocytes that vary in size. Mature/mature-ripening (stage IV) ovaries are partially ovulated and oocytes are released when squeezing the fish's sides. In this stage the ovary is at maximum development, but there are multiple stages of egg development occurring due to multiple spawning events in a given spawning season. Ripe (stage V) ovaries are relatively large, but more flaccid than a mature ovary. Ripe ovaries contain few vitellogenic oocytes of different sizes that are cream to yellow in color. This stage occurs in between spawning bouts until the end of spawning season.

Ovaries were teased apart for release of the oocytes from the dense matrix of connective stroma to allow examination of the oocytes. For intact pairs of ovaries, one of the ovaries was assessed for number of oocytes and that count multiplied by two to estimate the total number of oocytes (Stallsmith et al., 2015; Hodgskins et al., 2016). In cases where both of the ovaries were damaged during excision, the oocytes of both ovaries were counted and assessed.

Oocytes were grouped and arranged in a single layer on a microscope slide for imaging purposes. For a single ovary as many as eight pictures had to be made to capture all oocytes. Using Heins and Rabito's (1986) classification scheme, each stage of maturation was determined from the pictures. A modification of the scheme was used that excluded the latent stage ovaries in this study (Holmes et al., 2010). Therefore, stage 1 was deemed the early maturing stage followed by late maturing, mature and ripe, classified as stage 2, 3, and 4, respectively. In the early maturing stage, the oocytes are relatively small and translucent white in color. Late maturing oocytes are white to opaque in coloration. Mature stage oocytes have a distinct yellowish coloration. The counts for stages 3 and 4 in a female are the mature and ripe stages that have undergone vitellogenesis and are either close to being prepared or fully prepared for spawning and as such their combined number is an indicator of near-term spawning competence and are reported as clutch size (Heins and Rabito, 1986; Holmes et al., 2010; Stallsmith et al., 2015; Hodgskins et al., 2016). Ripe stage oocytes were visibly larger than previous stages, often ranging from yellow to dark yellowish-brown, with separation of the vitelline membrane and a

small yolk mass. Ripe oocytes are not present in the fish for very long, as they are quickly released upon reaching this stage of development. This stage of oocyte was not often found in collected specimens and was not reported as part of clutch size in this study. All oocytes were counted according to stage for the purpose of this study. Oocyte counts were performed from visual examination of digital photos for the 2012–2013 fish, and using EggHelper, a customized program to count developmental stages developed in Microsoft Visual Studio 2013 (Tarver and Tarver, 2014).

Data analysis

The null hypothesis of no significant differences among months within years in GSI, total oocytes and clutch size was tested by one-way ANOVA. Tukey HSD post hoc tests were performed on those tests showing significant p -values at $\alpha = 0.05$. All of these tests were done with the online Statistica calculator (Vasavada, 2016).

RESULTS

Reproductive development

Seven hundred seventy-seven specimens were collected over two 12-month periods in 2012–2013 and 2014–2015 (Table 1). Monthly collections are reported in sequence made, i.e. beginning and end of collections. River temperature for each collection is also reported in Table 1, ranging from a low of 6.6 °C in December, 2015 to a high of 27.1 °C in August, 2015. Especially in autumn and winter months the largest number of collected individuals was immature juveniles with little gonadal development. Females with enlarged ovaries containing maturing oocytes were observed in April – July of 2012, and in April – August of 2015 (Figure 2A, 2B). Stages 3 and 4 ovaries are indicators of the likely presence of mature/ripe ova

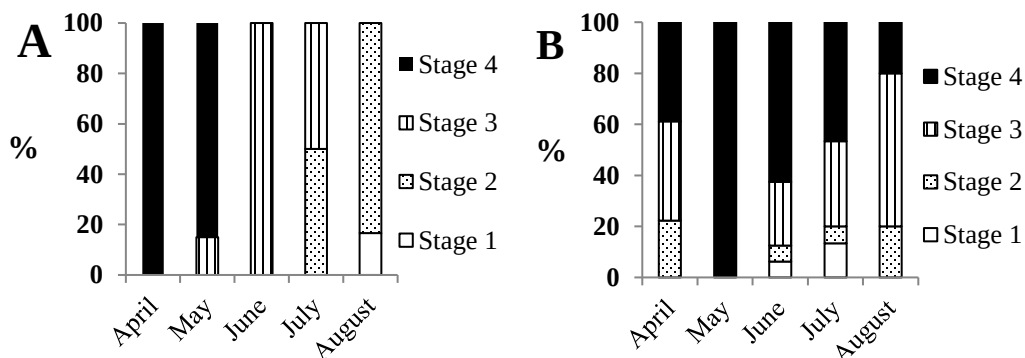


Figure 2. A) Maturation status of ovaries examined by month for 2012 by percentage. B) Maturation status of ovaries examined by month for 2015 by percentage. Oocyte maturation stages are indicated by different patterns within graph bars.

Table 1. Monthly fish collections in 2012-2013 and 2014-2015. Juveniles were fish near mature SL but lacking mature gonads. River temperature measured at time of fish collection is also reported.

Year/Month	Females	Males	Juveniles	Temperature, °C
2012-2013				
April	9	10	2	20.6
May	20	2	0	22.0
June	12	5	23	25.6
July	5	8	4	26.4
August	6	2	31	23.3
September	9	5	19	21.4
October	4	6	8	19.2
November	24	21	17	13.0
December	4	4	15	8.0
January	7	3	22	7.4
February	4	1	28	8.8
March	2	2	7	15.8
2014-2015				
September	2	4	59	21.3
October	7	5	17	18.0
November	1	3	19	11.7
December	7	8	13	6.0
January	1	10	27	9.6
February	8	10	34	10.6
March	8	12	17	15.5
April	18	23	13	17.8
May	2	1	2	23.4
June	17	14	2	28.3
July	15	5	0	25.5
August	5	5	32	27.1

classifying a female as reproductively active. In 2012, stage 4 ovaries were found only in April and May. The number of oocytes found in an individual ranged from 19 in July, 2012, to 1288 in June, 2012, with monthly means ranging from 281 in August, 2015, to 972 in June, 2012 (Figure 3). The average number of oocytes per fish was higher in 2012 than in 2015 for all months except for May which was close to 560 oocytes in both years. Stage 1 oocytes were typically the most abundant, although some months had stage 3 oocytes as the most abundant group. Stage 3 oocytes were especially numerous in May and June, 2012, and were present both years in April through August. Few stage 4 oocytes were found in any month, typically only 2% of oocytes per month. A one-way ANOVA was used to determine if significant differences existed between monthly counts of average total oocytes

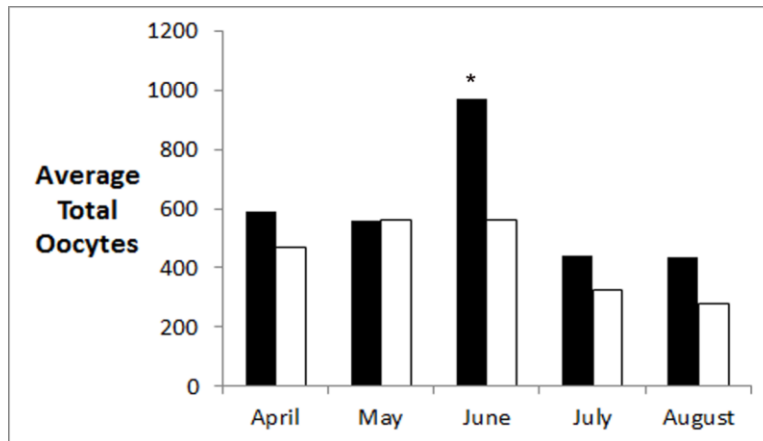
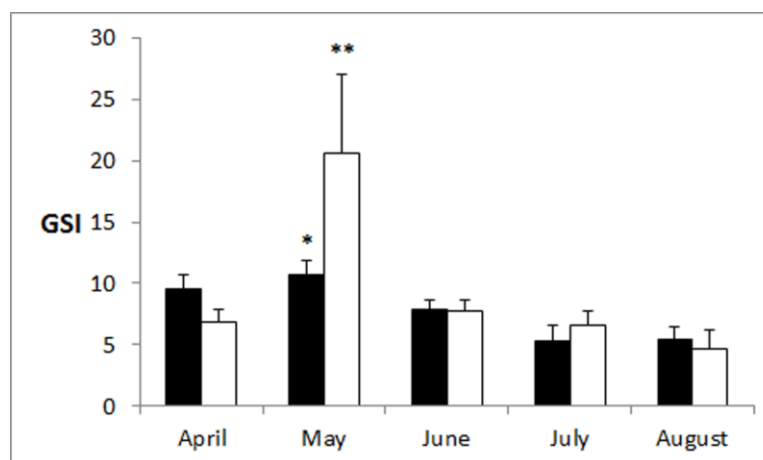


Figure 3. Monthly average total oocytes found in 2012 (black bars) and 2015 (open bars). Asterisk indicates significant difference from the other four months of 2012 at $p < 0.05$.

in each year. For 2012 data a significant difference was found between months with $F_{(2,4)} = 8.72$, $p < 0.001$. Testing these results with Tukey HSD showed June to be significantly different from each of the other four months with $p < 0.01$. Similar testing found no significant differences between months in 2015.

For both years the peak female average monthly GSI value was in May with elevated values in April and June and a fall off into July and August (Figure 4). For 2012 data a one-way ANOVA was used to determine if significant differences existed between monthly GSI averages. The test showed significant differences existed with $F_{(2,4)} = 3.30$, $p = 0.02$. A post-hoc test of these results with Tukey HSD identified May as significantly different from the other four months with $p < 0.05$. The same test for 2015 GSI monthly averages indicated significant differences between months with $F_{(2,4)} = 5.32$, $p = 0.001$. A post-hoc Tukey HSD test identified May as significantly different from other months with $p < 0.01$.

Figure 4. Monthly average female GSI with standard error bars found in 2012 (black bars) and 2015 (open bars). Single asterisk represents significance of higher GSI at $p < 0.05$ compared to other months in 2012; two asterisks represents significance of higher GSI at $p < 0.01$ compared to other months in 2015.



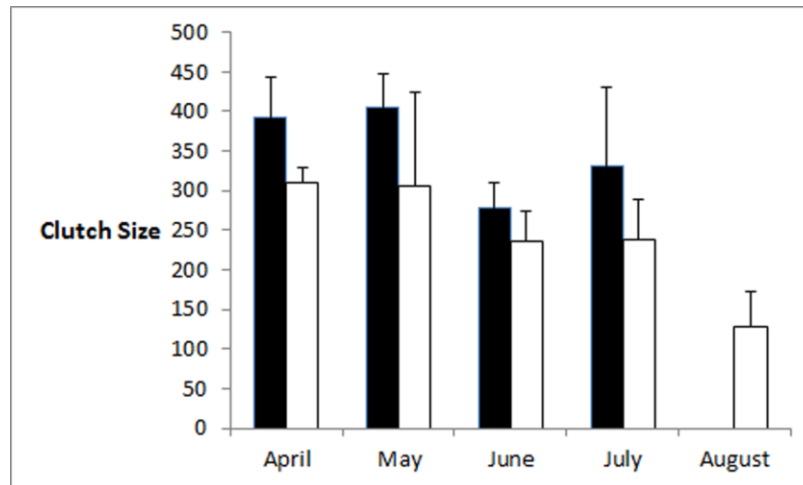


Figure 5. Monthly average clutch size with standard error bars for 2012 (black bars) and 2015 (open bars). No significant differences were found between months in either year.

Monthly average male GSI in 2012 ranged from 0.63 in May ($n = 5$) to 1.64 in April ($n = 2$). A one-way ANOVA test for significant differences between months showed significance with $F_{(2,4)} = 2.91$, $p = 0.05$ but a post-hoc Tukey HSD test found no significant differences between months. In 2015 monthly average male GSI ranged from 0.42 in July ($n = 5$) to 0.99 in April ($n = 24$). Because only one sexually mature male was found in May, the month was excluded from a one-way ANOVA test for significant differences between months. This one-way ANOVA test for differences between the remaining four months resulted in $F_{(2,3)} = 2.08$, $p = 0.12$, indicating no significant difference.

Monthly average clutch size is shown in Figure 5 along with standard error bars. One-way ANOVA tests of monthly average clutch size for each year's data did not support a finding of significant differences in clutch size between months for either 2012 or 2015. Average clutch sizes were largest in April and May of each year with values between 300 and 410 followed by decline through July and August.

DISCUSSION

We found the Scarlet Shiner to spawn primarily in late spring, with reproductive effort trailing off into mid-summer. Individuals with mature ovaries were found only in April through July for one year, and April through August for the other year; the same pattern held for individuals with mature oocytes.

Female GSI each year had a significant peak in May. A major determinant of the onset and peak of Scarlet Shiner spawning is likely to be the availability of spawning sites maintained by nest associates. Spawning aggregations of Scarlet

Shiners in the Flint River have been observed in close association with the nests of Longear Sunfish (J. Mann, personal communication). The strong river pulses of winter and early spring in the Flint River typically diminish by April (Hodgskins et al., 2016). It is unlikely that Longear Sunfish exhibit nesting behavior and build their nests in the sustained high water of such pulses. Rivers with higher water levels are also colder. For instance, the Flint River was warmer on collection days in April and May of the near-drought year 2012, 20.5° and 22° respectively, than in April and May of the wetter year 2015, 18° and 19° respectively.

Spawning for most North American cyprinids takes place in spring and summer months (Boschung and Mayden, 2004). Different patterns of GSI and oocyte development exist between single and fractional spawners (Winemiller and Rose, 1992). In the case of fractional spawners like the Scarlet Shiner, oocyte development is staggered allowing for stages from latent to ripe to be present at the same time, and vitellogenesis occurs throughout the breeding period. Unlike single spawners, multiple spawners undergo a gradual decline in GSI over the spawning season before reaching a point of gonadal quiescence as was found with the Scarlet Shiner (Figure 4) (Heins and Rabito, 1986).

The timing of reproduction by a stream fish is crucial to its own success in a given stream, and must also affect other species' success in ways crucial to community structure (Matthews and Marsh-Matthews, 2017). The Flint River flows year-round, but can vary inter-annually in the timing and severity of flood pulses in late winter and early spring (Hodgskins et al., 2016). The Scarlet Shiner begins spawning in April when the threat of such flood pulses has diminished, and continues spawning as late as July or even August which would allow some reproductive success in years when flood pulses occur in April and May and likely carry away eggs and larvae.

Compared to other studied cyprinid species in the Flint River, the Scarlet Shiner was found to be a relatively late spawner. Research on the Blotched Chub (*Erimystax insignis*) in the Flint River (Stallsmith et al., 2015) established that species' spawning season to be March to May. Research on the Silver Shiner (*Notropis photogenis*) has shown the spawning season to be February to April and likely dependent on river pulses to disperse eggs and larvae (Hodgskins et al., 2016), while the Whitetail Shiner (*Cyprinella galactura*) spawning season is May to August (B. Stallsmith, unpublished data). The Scarlet Shiner is similar to the Whitetail Shiner in that both show strong sexual dimorphism with "bourgeois" males competing for females in defended territories with later spawning during warmer water temperatures, and both species produce markedly fewer oocytes per female than Blotched Chubs and Silver Shiners.

Scarlet Shiner life history can be defined by what Winemiller defined as “opportunistic”, with larger and more conspicuous males, small clutches, and early maturity (Winemiller, 1992). The relatively long observed breeding season of Scarlet Shiners, April to July or August, is consistent with the production of repeated small clutches by females. This breeding season seems to be a well-defined reproductive niche, different from three other cyprinid species in the Flint River whose reproductive strategies have been studied.

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