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Reproductive Schedule of the Silver Shiner (Notropis photogenis) in the Flint River of Alabama

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Abstract

Many river-dwelling species of fish are dependent upon and stimulated by fluctuations in river flow for successful reproduction. This is especially true of pelagophils, a reproductive guild whose eggs and larvae require free drifting on river currents for several days. *Notropis photogenis* (Silver Shiner) is a rheophilic species with a broad distribution from Ontario to the southeastern United States including northern tributaries to the Tennessee River in Alabama. Little is known of its reproductive biology. The purpose of this study was to describe aspects of reproductive biology such as timing and pattern of ovarian development and oocyte maturation of *N. photogenis* in the Flint River of Alabama. We investigated whether and how abiotic cues such as river discharge and temperatures were related to ovulation and spawning. Monthly fish collections were made from August, 2011, to July, 2013. From these collections monthly gonadosomatic index (GSI) was evaluated, along with the status of ovarian maturation, oocyte maturation and size, and oocyte counts to establish fecundity and clutch size. Median monthly river discharges in cubic feet per second for 1999–2015 were obtained from the U.S. Geological Survey database. Observations over two years showed associations between daily mean discharge and months of peak GSI (February–April). Mean GSI peaked in March of both 2012 and 2013 when median flow was approximately 600 cfs. Large synchronous spawning events appear to occur during times of steady substantial discharge increases but after peaks of discharge > 3000 cfs.

Keywords

cyprinid, freshwater ecology, synchronous spawning

Cover Page Footnote

Josh Mann, Kara Million and Crissy Tarver helped with the fieldwork for this project. Comments from two reviewers much improved the manuscript.

INTRODUCTION

Notropis photogenis (Silver Shiner) is a fluviatile, rheophilic species with a broad distribution from Ontario, Canada, to the southeastern United States including northern tributaries to the Tennessee River in Alabama (Page and Burr, 2011; Stallsmith and Thompson, 2012). The status of *N. photogenis* in tributaries of the Tennessee River in north Alabama has been unclear, as the species has only been occasionally collected since the 1960s (reviewed in Stallsmith and Thompson, 2012). It has been our experience that the species is found in deeper, faster flowing sections of rivers, making it more difficult to collect especially during elevated river flow. In Alabama the conservation status of the species is Moderate Concern (Alabama Department of Conservation and Natural Resources, 2008), which may accurately reflect the species' dependence on the dwindling number of free-flowing rivers such as the Flint River.

For such a wide-spread species, little is known of its reproductive biology. Alabama is at the southernmost edge of the species' distribution. Only one other similar study seems to have been made of this species, in the Thames River of Ontario, the northernmost population (Baldwin, 1983). It has been suggested that *N. photogenis* may move from its normal foraging habitat to spawn in waters greater than 1 m deep (Parker and McKee, 1984), though the necessary habitat has not been defined to date. There is limited knowledge about details of the species' reproduction such as the optimal temperatures required for successful spawning, or if the species spawns communally or in pairs. In Ontario, Canada, *N. photogenis* spawning appears to occur in mid-June when water temperatures average 23°C (Parker & McKee, 1984). Many river-dwelling species of fish are dependent upon and stimulated by fluctuations in river flow for successful reproduction. This is especially true of pelagophils, a reproductive guild whose propagules (eggs and larvae) require free drifting on river currents for several days before finding suitable habitat to transition to a free swimming stage. Spawning typically occurs during elevated flows of spring run-off or summer storms which transport the ichthyoplankton downstream.

The purpose of this study was to describe aspects of the reproductive biology such as timing and pattern of ovarian development and oocyte maturation of *N. photogenis* through sampling fish over two reproductive seasons in the Flint River in Alabama. Additionally, we examined whether and how abiotic cues such as river discharge and temperatures may be related to ovulation and spawning as measured by gonadal maturation, oocyte maturation, and oocyte size. Inter-annually, the Flint River has varying mean Richards-Baker Flashiness Index (RBFI) values (Kelly Hodgskins, unpublished data) showing it is prone to rapid

changes in discharge related to storm runoff (Fongers, Manning, & Rathbun, 2007). We were interested whether such changes in discharge are related to the spawning of *N. photogenis*.

METHODS

Study Site

All fish were collected in Madison County, AL from three sites in a 15 km section of the Flint River located east of Huntsville: Oscar Patterson Road, 34° 52' 51" N, 86° 28' 22" W; Winchester Road, 34° 49' 20" N, 86° 28' 57" W; and Mount Carmel, 34° 48' 24" N, 86° 28' 22" W (Figure 1). The Flint River drains 141,640 hectares of Madison County, Alabama and Lincoln County, Tennessee (Abdi et. al, 2009), and its major branches are a total of 562 km long. The river rises in southeastern Lincoln County, TN, and flows south through Madison County, AL, into the Tennessee River southeast of Huntsville, Alabama. 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 Tuscumbia limestone and Fort Payne chert from the Mississippian Period. 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. During periods of low flow, bars of cobble are exposed proximal to the center of the channel. A low to moderate amount of aquatic vegetation is present near the bars and banks in summer months. Riparian growth meets the river banks and extends approximately 50 meters away from the bank on either side of the channel. According to the National Consortium on Remote Sensing in Transportation, the Flint River watershed is comprised of 1471 square km of predominantly agricultural land. Rapid development has been occurring in the Huntsville, AL region since 1984. Data suggest that annual increases seen in stream flow are correlated with land development in the area (Goodman, 2004).

Fish Sampling

Fish were collected monthly from August of 2011 through July of 2013 except in December 2011, January 2012, and December 2012. Collections were made using a seine net (3.5 m length, 1.2 m depth, and a 3 mm mesh) or a cast net (radius of 1.37 m and a 6 mm mesh). All fish were euthanized on site using 2 ml of (1:10) clove oil: 95% ethanol added to 350 ml river water (Wong, 2014). Euthanized fish were placed in 10% phosphate buffered formalin for 24 hours to promote tissue fixation as well as temporary storage (Jordan, 1902). River temperature was recorded on each trip using a digital thermometer.

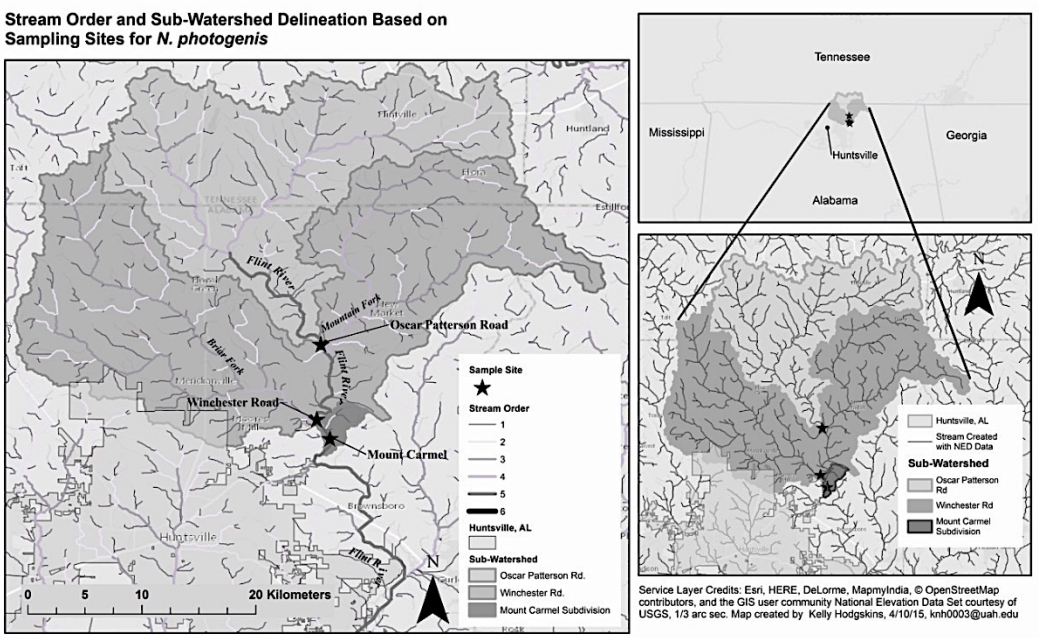

Figure 1. Collection site locations with calculated stream order and delineated sub-watersheds. Sub-watershed shading shows the additional contributing areas of sub-watershed inputs based on collection sites.

Laboratory Analysis

Digital calipers were used to measure standard length (SL) of each specimen to the nearest 0.01 mm. An Explorer OHAUS digital balance was used to obtain gross body mass to the nearest 0.0001 g after excess fluid was blotted from the fish's body. Gonadal mass was obtained to the nearest .00001 g after excess surface fluid was blotted away using paper towels. All gonadal tissue was stored in 10% phosphate buffered formalin during the study. Sex was determined at the time of gonadal excision due to low sexual dimorphism. Individuals ≤ 55 mm were classified as juveniles and sex was not determined. Ovipositors were present only when females were ripe and in breeding condition. Excised gonadal tissue and ova were imaged with an Olympus SZX7 dissecting microscope equipped with a 12 megapixel digital camera controlled with the cellSens software package.

A modified method originally developed by Núñez and Duponchelle (2009) was used to assess ovarian maturation. Ovaries were divided into five stages of maturation (Figure 2). 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

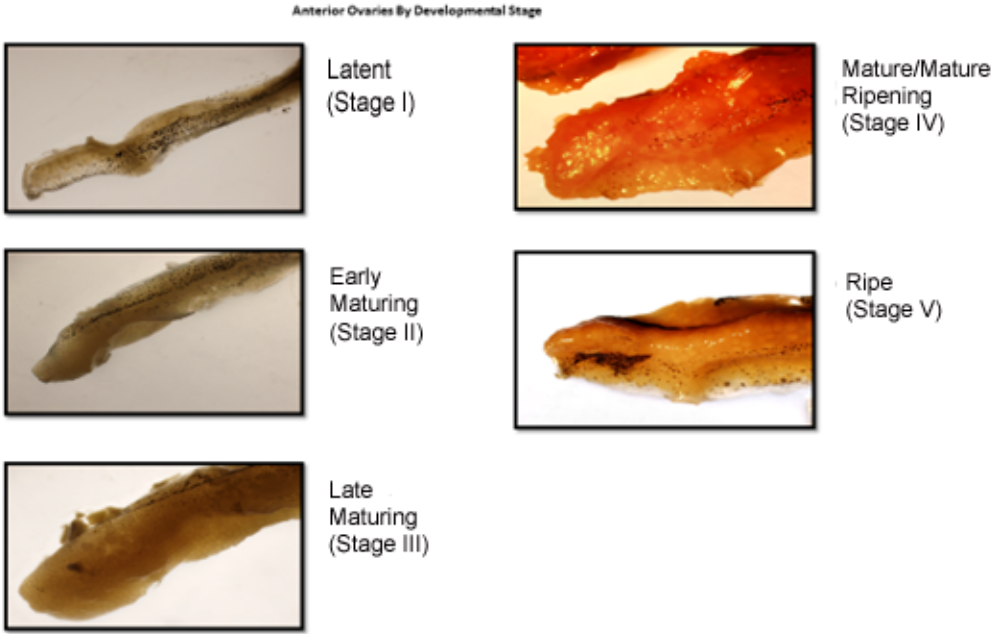

Figure 2. Anterior ovarian heads imaged at 1.6X total magnification. Ovaries are presented in their five stages of development.

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. Ripe (stage V) ovaries are relatively large, but more flaccid than a mature ovary. Ripe ovaries contain different sizes of developing vitellogenic oocytes that are cream to yellow in color. This stage occurs in between spawning cycles until the end of spawning season. Latent oocytes were not included in this study. Early maturing (stage I) oocytes are distinguished by their small size, less than half the diameter of a mature oocyte, and previtellogenic. For late maturing (stage II) oocytes, the diameter increases and yolk granules begin to form in the early stages of vitellogenesis. The formation of the nuclear envelope can be seen and their size is greater than half the mean diameter of mature ova. Mature (stage III) oocytes are in late vitellogenesis and filled with yolk globules. The oocytes are yellow to cream in color and opaque. The vitelline membrane is dividing from the yolk. Ripe (stage IV) oocytes are the largest, yellow to dark orange in color, and have vitelline membranes that are completely separated from the yolk mass.

Using 21-gauge hypodermic needles, developing oocytes were liberated from the ovarian tissue and arranged into a single layer on a Syracuse watch glass. Images were taken at a total magnification of 1.6X. When the number of oocytes

exceeded one frame, multiple frames were imaged. Digital images were assessed to categorize oocytes into stages of maturation (Figure 3).

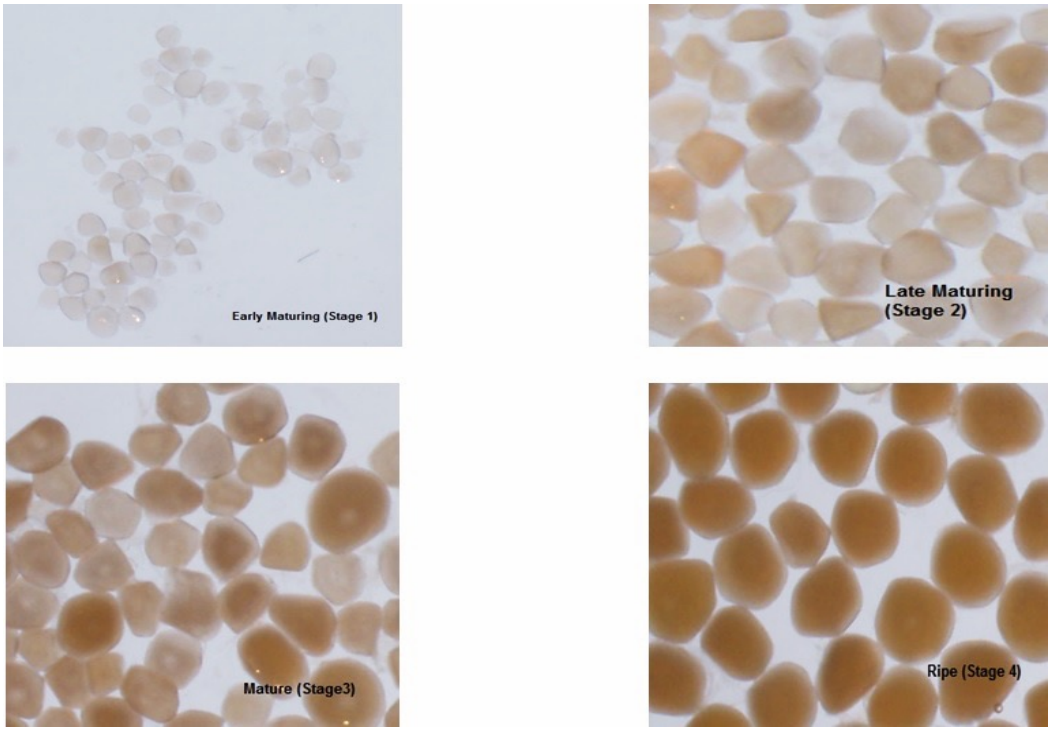

Figure 3. Four stages of egg maturation for *N. photogenis*. Images were taken at 1.6x total magnification. Latent eggs were not included in this study.

Early maturing, late maturing, mature, and ripe oocytes were counted by stages and the total number of oocytes was calculated for one ovary per female for fish with a GSI > 7. Additionally, November 2012 collection females with a GSI ≥ 3 were included in the study for the purpose of having more sample points and oocyte stage variety. The assumption was made that doubling the total number of oocytes calculated will yield the total number of oocytes for both ovaries (Stallsmith et al., 2015). Total oocyte counts by developmental stage and complete clutch size was determined. Clutch size was determined by counting the total number of stage IV oocytes present in advanced maturation (stage III, late maturing) ovaries (Heins, 1995). In cases where both of the ovaries were damaged during excision, the oocytes of both ovaries were counted and assessed, then divided by two to represent the contents of one ovary for statistical analysis. Oocyte counts were performed using EggHelper (Tarver, 2014), a customized program developed in Microsoft Visual Studio 2013. Results were confirmed using cellSens software. Diameters of 10 oocytes per stage, per image taken, were measured and the monthly average for each oocyte stage was calculated. Ripe

(stage 4) oocytes from late maturing ovaries were used to determine maximum diameter (Heins, 1995).

Statistical Analysis

Reproductive investment was examined using average monthly gonadosomatic index values (GSI) for adult males and females using the equation: GSI = (Gonadal mass (g) / (gross body mass (g) – gonadal mass (g))) X 100. Shapiro-Wilk goodness-of-fit tests were used to assess normality of distribution, and homogeneity of variance was evaluated using Levene's test on the monthly GSI data. Because those tests did not find homogeneity of variance in the data, one-way Welch's ANOVAs with post hoc Games-Howell tests were chosen to determine if statistically significant differences between monthly GSI values existed between monthly samples of both males and females.

Gonadosomatic index and egg diameter were plotted by year of collection to identify possible synchronized spawning events. Apparent synchronized spawning events were identified as any sampling dates where GSI or oocyte diameter decreased by at least two times the standard error from the sampling date immediately preceding it (Durham & Wilde, 2014). Female GSI was plotted against date of collection and mean river discharge (cfs) at time of collection to discern possible influences of discharge on gonadal development. A nonparametric 2 X 1 Pearson χ^2 analysis was used to assess for a significant difference between the total number of males and total number of females in monthly collections. An independent t-test was conducted to compare stage IV oocyte diameters between collection years 2012 and 2013.

Environmental Influences

Median monthly river discharge data from 1999 to 2015 were obtained from USGS river gauge #03575100 located downstream in Brownsboro, AL to determine the months of peak discharge (USGS, 2015). Using Microsoft Excel (2013), female GSI was plotted against date of collection and mean river discharge (cfs) at time of collection to discern possible influences of discharge on gonadal development.

RESULTS

Ovarian Development, Oocyte Diameters, Fecundity, and Clutch Size

Excised ovaries were assessed for developmental stage and categorized into one of five stages by month and year (Figure 4). April, 2012, and May, 2013, were the only two months in which all ovaries sampled were categorized as stage V, ripe ovaries. Stage IV ovaries, mature/mature ripening, were present in 100% of females sampled for February and March, 2013. Maturation to stages IV and V occurred a full month later in 2013 than it did 2012. Stage III, late maturing ovaries, were present in October and November, 2011, and in 100% of the females from February, 2012. No stage III ovaries were found in females in 2013. Stage II, early maturing ovaries, were found in 100% of females from September, 2011, and November, 2013. Stage I, latent ovaries, were found in 100% of females in August, 2011; May, 2012 – August, 2012; January, 2013; and June, 2013.

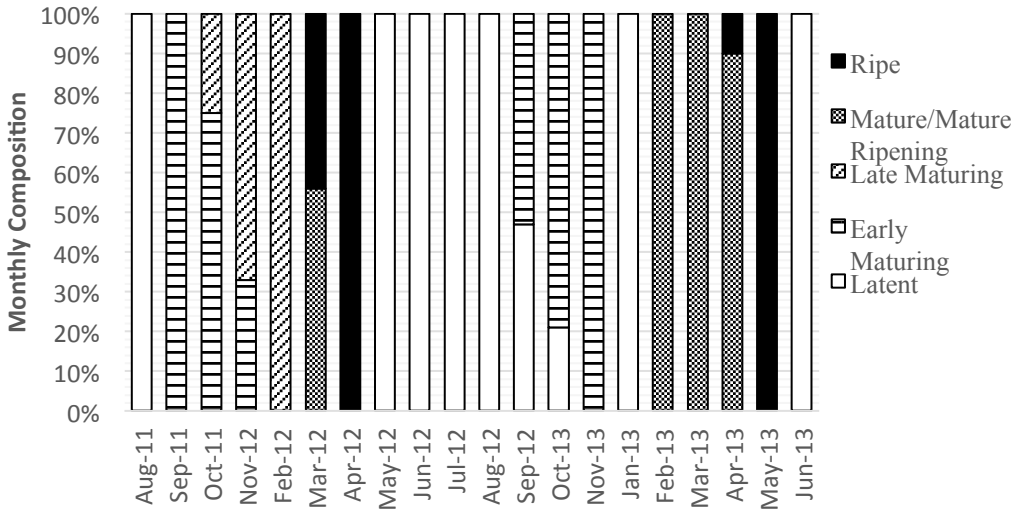

Figure 4. Ovarian stages by month and year of collection.

Of the 170 females sampled, 17 met the criterion of having ovaries at stage III, the stage for estimating clutch size (Table 1). Complete clutch sizes were determined for these 17 females. The smallest fecund female measured 75.2 mm in standard length, carried an estimated total count of 16,220 oocytes, a clutch size of 396 oocytes, and a GSI of 15. The largest fecund female measured 92.9 mm in standard length, carried an estimated total count of 7,492 oocytes, a

clutch size of 274 oocytes, and a GSI of 12.4. The smallest total oocyte count recorded was 4,998 oocytes and the largest 18,230 oocytes, in fish measuring 86.3 and 83.3 mm respectively. The largest clutch recorded was 1,226 oocytes for an April 2013 fish with a GSI of 11 and an SL of 88.1 mm. Out of the 170 females the highest GSI recorded was 45 and a total of 10 fish had a GSI ≥ 20. Total fecundity was also lower in 2013 than 2012 for all months examined (Table 2).

Table 1. Clutch sizes based on complete oocyte count by stage for one ovary, determined for Stage III-Late/Advanced Maturation ovaries as recommended by Heins (1995).

Year/Month	Stage 1	Stage 2	Stage 3	Stage 4	Oocyte Count	SL(mm)	GSI
2012							
February	4952	2926	37	–	7915	87.3	10
February	2608	1747	29	–	4384	87.9	8
February	5279	3577	259	–	9115	83.3	9
February	2753	2020	488	–	5261	87.4	12
February	2514	1546	472	903	5433	91.6	15
February	2508	1943	281	–	4732	92.6	11
February	4438	3691	333	–	8462	90.9	10
February	1718	789	1102	137	3746	92.9	12
March	1415	2544	1388	293	6640	86.9	9
March	657	3512	2033	198	8110	75.2	15
March	1207	650	61	97	3395	90.5	13
March	1022	781	1000	560	3363	85.0	20
2013							
February	1870	1779	165	–	3814	83.4	8
February	1986	1993	256	–	4235	87.1	8
February	1049	1921	141	–	3111	87.2	7
April	807	973	308	676	2764	88.1	11
April	573	925	388	613	2499	86.3	15
Mean					5116 (S.E. 506)		

Stage IV oocytes were present in February and March of 2012 (Figure 5) and in March, April, and May of 2013. Stage I, II, and III oocytes were present in five months (February, March, April, May, November). Stage I and II oocytes were present in all seven months examined. Mean monthly oocyte counts (±SE) by developmental stage (Table 2) showed variation between months and years. No stage IV oocytes were present in the November or January samples but were present in February and March of 2012 and February, March, April, and May in 2013. The highest counts of stage IV oocytes were in March, 2012 (674 ± 203) and March, 2013 (708 ± 78). Stage III oocytes were present in greatest numbers in March, 2012 (1171 ± 224) and March, 2013 (764 ± 110). The lowest number of stage III oocytes found (61 ± 19) was in November, 2012. February of both 2012 and 2013 also had stage III counts < 300.Stage II oocytes were present in all months examined. Stages I and II were present in all months of interest. Stage I

Table 2. Mean monthly oocyte counts (±SE) by developmental stage and calculated total fecundity for months for which mature females were available. November 2012 was included for evaluation of GSI's < 7%, for all other months specimens were required to have a GSI of ≥ 7%.

Month/Year Individuals	Mean # Stage I Oocytes	Mean # Stage II Oocytes	Mean # Stage III Oocytes	Mean # Stage IV Oocytes	Total Fecundity (one ovary)
February 2012 (n = 8)	3579 ±474	2493 ±337	271 ±70	129	6472
March 2012 (n = 9)	1511 ±259	1619 ±344	1171 ±224	674 ±203	4848
November 2012 (n = 23)	2487 ±237	1503 ±78	61 ±19	0	3987
February 2013 (n = 3)	1635 ±295	1898 ±63	187 ±35	0	3720
March 2013 (n = 7)	863 ±181	913 ±138	764 ±110	708 ±78	3391
April 2013 (n = 10)	523 ±63	724 ±84	334 ±52	604 ±50	2197
May 2013 (n = 1)	415	946	360	648	2369

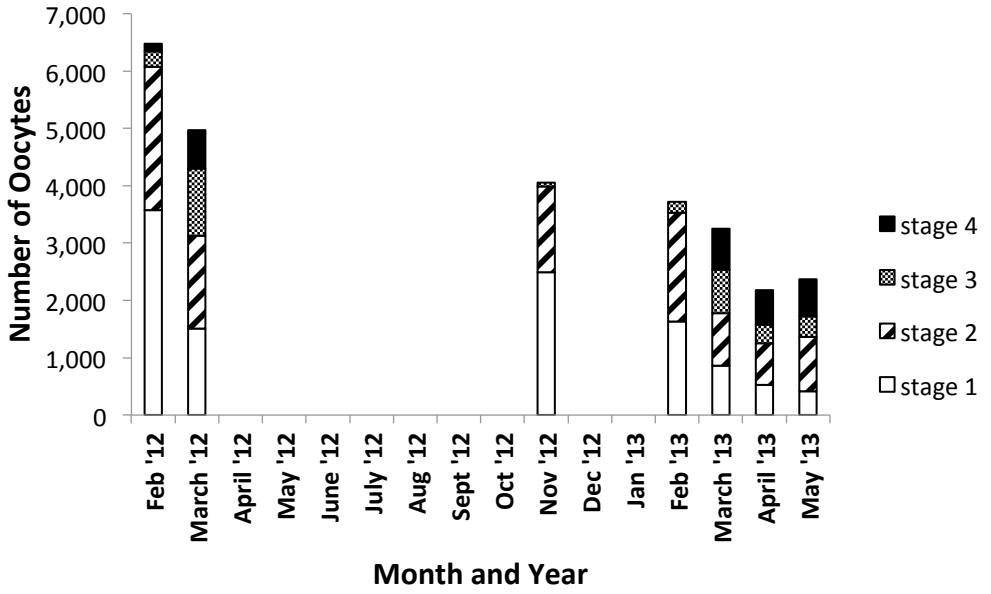

Figure 5. Total counts of oocytes by oocyte development (stages I – IV).

oocytes were present in greatest numbers in February of 2012 (2493 ± 337) and 2013 (1898 ± 63). The greatest number of stage I oocytes was found in February, 2012 (3579 ± 474). With the exception of stage IV oocytes in March, 2012, all stage counts for 2013 were lower than 2012.

The monthly average diameter for each oocyte stage was calculated using pooled data (Table 3). No stage 4 oocytes were found in February 2013, but

Table 3: Mean diameters (±SE) by monthly oocyte developmental stage for adult *N. photogenis* females in months of interest.

Month/Year	Mean Diameter Stage I (mm)	Mean Diameter Stage II (mm)	Mean Diameter Stage III (mm)	Mean Diameter Stage IV (mm)
February 2012	0.390 ±0.02	0.630 ±0.01	0.783 ±0.02	0.853
March 2012	0.413 ±0.01	0.624 ±0.01	0.801 ±0.02	1.040 ±0.04
November 2012	0.395 ±0.01	0.524 ±0.01	0.599 ±0.01	n.a..
February 2013	0.484 ±0.02	0.548 ±0.01	0.689 ±0.02	n.a.
March 2013	0.457 ±0.01	0.628 ±0.01	0.767 ±0.04	1.186 ±0.02
April 2013	0.451 ±0.01	0.600 ±0.01	0.741 ±0.01	0.966 ±0.03
May 2013	0.505	0.664	0.713	1.173

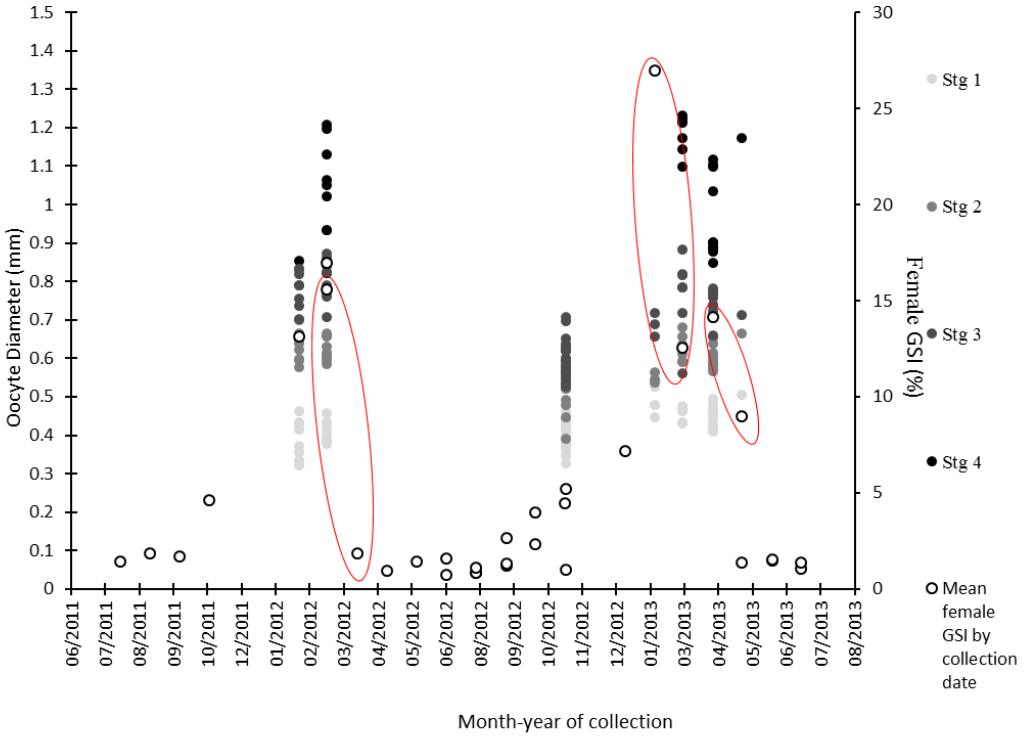

Figure 6. Identification of synchronized multiple spawning events (in red ovals) for female *N. photogenis* based on date of collection, oocyte diameter, and female GSI.

February 2012 oocytes had a mean diameter of 0.853 mm. March of 2012 and 2013 both yielded stage 4 oocytes with diameters > 1 mm. March showed the greatest stage 4 oocyte diameters out of all months. No stage 4 oocytes were found in April 2012, but 2013 yielded diameters > 1 mm. Mean diameters (±SE) by developmental stage for all collections are as follows, stage I (0.442 ± 0.02 mm), stage II (0.603 ± 0.02mm), stage III (0.728 ± 0.02mm), and stage IV (0.95 ± 0.05 mm). Ripe oocytes from 2012 had a mean diameter of 1.02 mm ± 0.14 and ripe oocytes from 2013 had a mean diameter of 1.06 mm ± 0.14. An independent t-test ($t(26) = -1.047$, $p = 0.31$) showed no significant differences in mean diameters between 2012 (mean = 1.02 mm, SD = 0.14, n = 10) and 2013 (mean =1.11 mm, SD = 0.26, n = 18) for ripe oocyte diameters. Oocyte size did not vary between the years of collection. Figure 6 identifies probable synchronized multiple spawning events for female *N. photogenis* based on date of collection, oocyte diameter, and female GSI.

Reproductive Schedule

The average monthly GSI was evaluated for male and female *N. photogenis* collected from August, 2011 to July, 2013 (Figure 7). Thirty four out of thirty six collections contained females with a total of 170 females sampled for GSI. GSI values began to rise in November, 2011 and November, 2012 for both males and females. In March of 2012, the mean GSI value for females peaked at 16.5 (SE = 1.75) and in March of 2013, mean GSI peaked at 27 (SE = 3.44). The mean GSI for males peaked in February of 2012 at 1.1 (SE = 0.08) and in 2013, mean GSI for males peaked in March at 1 (SE = 0.079). A large decrease was seen in male and female GSI values from March to April of both 2012 and 2013, and also from April to May in both years. For females a statistically significant difference in monthly GSI was revealed by ANOVA results, $F_{20, 169} = 33.83$, $p < .001$, and for males, $F_{19, 188} = 6.96$, $p < .001$ (indicated by letters over monthly bars, Figure 7).

Environmental Relationships

Discharge typically peaked in December and remained high until April when it begins to decline rapidly before reaching mid-June when discharge drops to less than 300 cfs (Figure 8). The period of low flow is from June to the beginning of November. For months of peak median flow (February–April), the mean temperatures at times of collection were 12 – 18 °C respectively. Discharge began to increase in November 2011 and December 2012 then began to taper off quickly in April of both 2012 and 2013; however the decline was more gradual in April 2013 with discharges > 2000 cfs occurring more frequently through April and into July. Peaks in mean female GSI and oocyte size by collection date

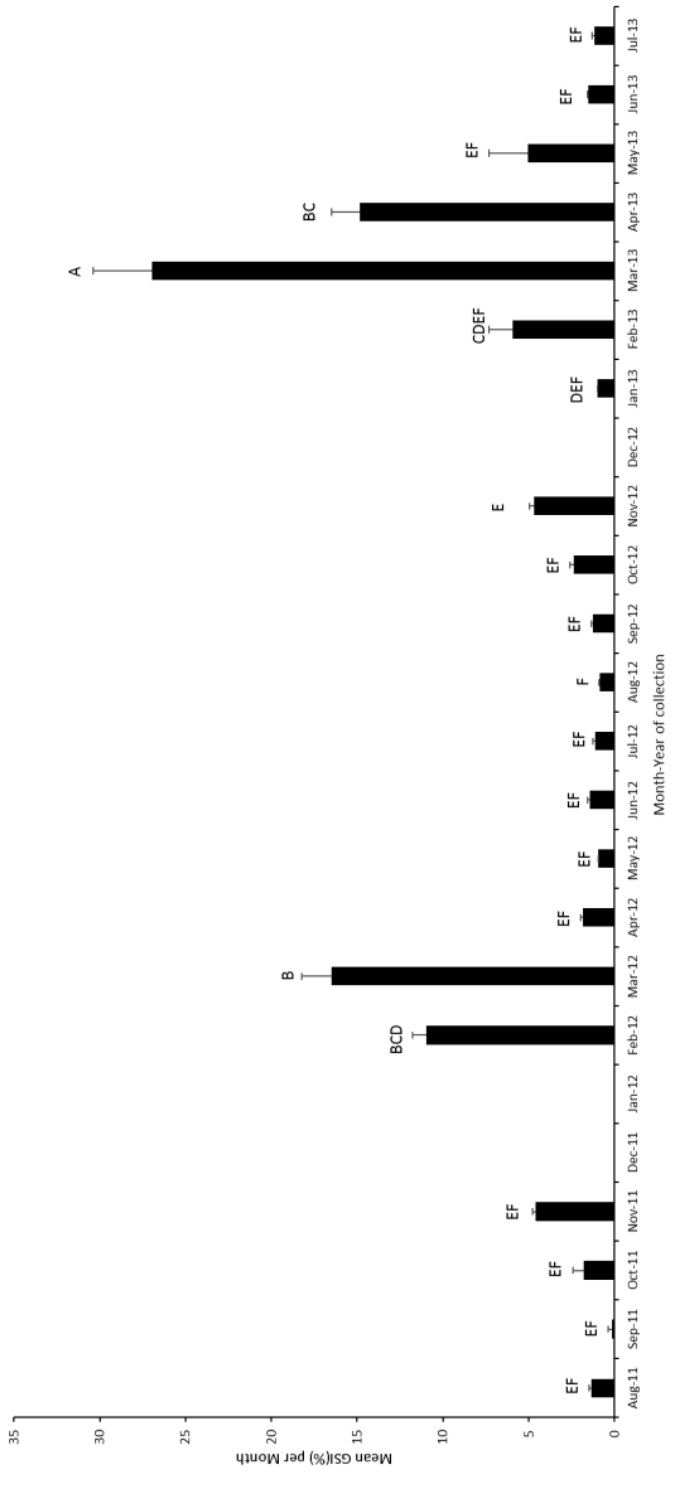

Figure 7. Mean monthly GSI values for females with results of post-hoc Games-Howell test of Welch's ANOVA tests. Statistically significant groupings of months are indicated by letter in the middle column. Multiple letters for a month indicate overlaps with two or more groupings for a month. Months when no females were collected are omitted.

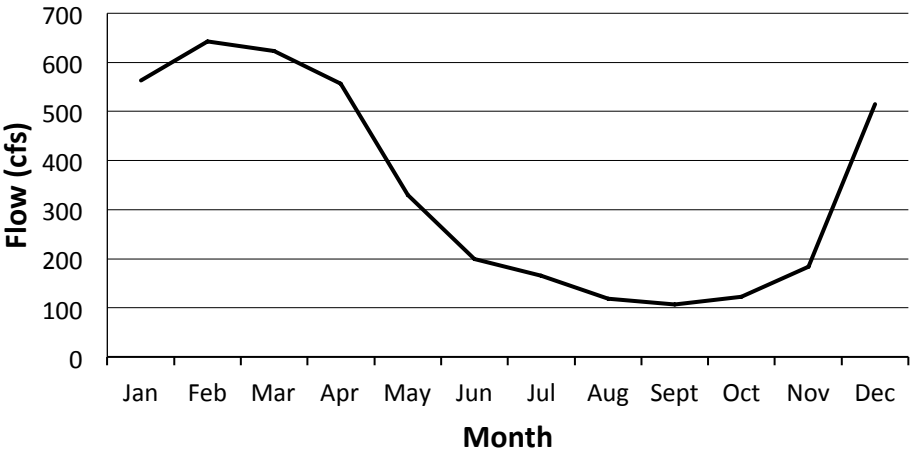

Figure 8. Median discharge (cfs) of the Flint River from 1999 – 2015. USGS river gauge #03575100 is located downstream of collection sites in Brownsboro, AL. Data courtesy of USGS.

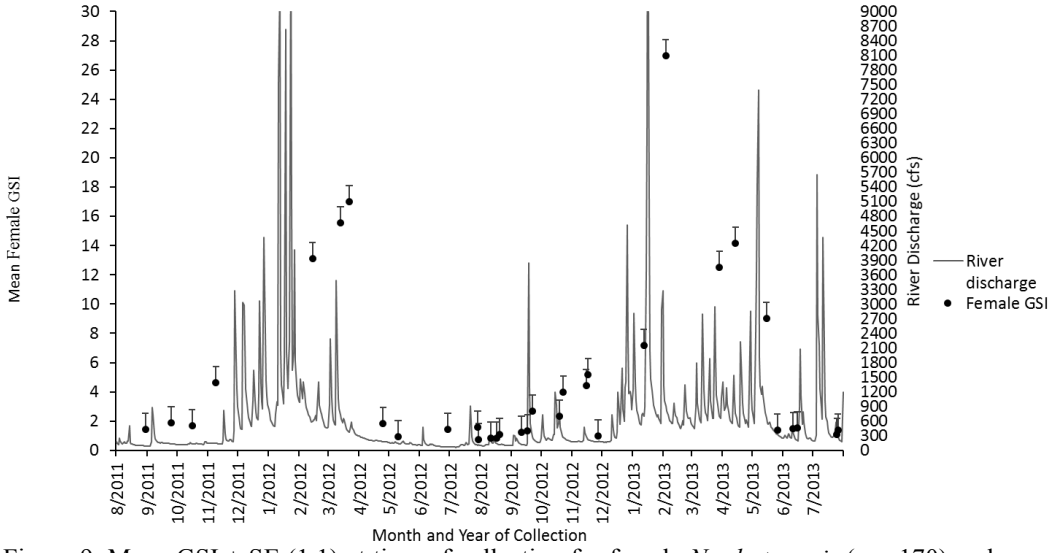

Figure 9. Mean GSI ± SE (1.1) at time of collection for female *N. photogenis* (n = 170) and mean daily discharge on the Flint River between August 2011 and July 2013.

appeared after river discharges of from 3000–9000 cfs (Figure 9). Female GSIs were lowest during periods of low discharge.

A third order polynomial regression of river temperature against monthly GSI yielded an $R^2 = 0.541$ ($p < 0.001$) for females and an $R^2 = 0.623$ ($p < 0.001$) for males (Figure 10). Other possible cofactors such as precipitation, day length, dissolved oxygen, turbidity, and air temperature were not evaluated in this study.

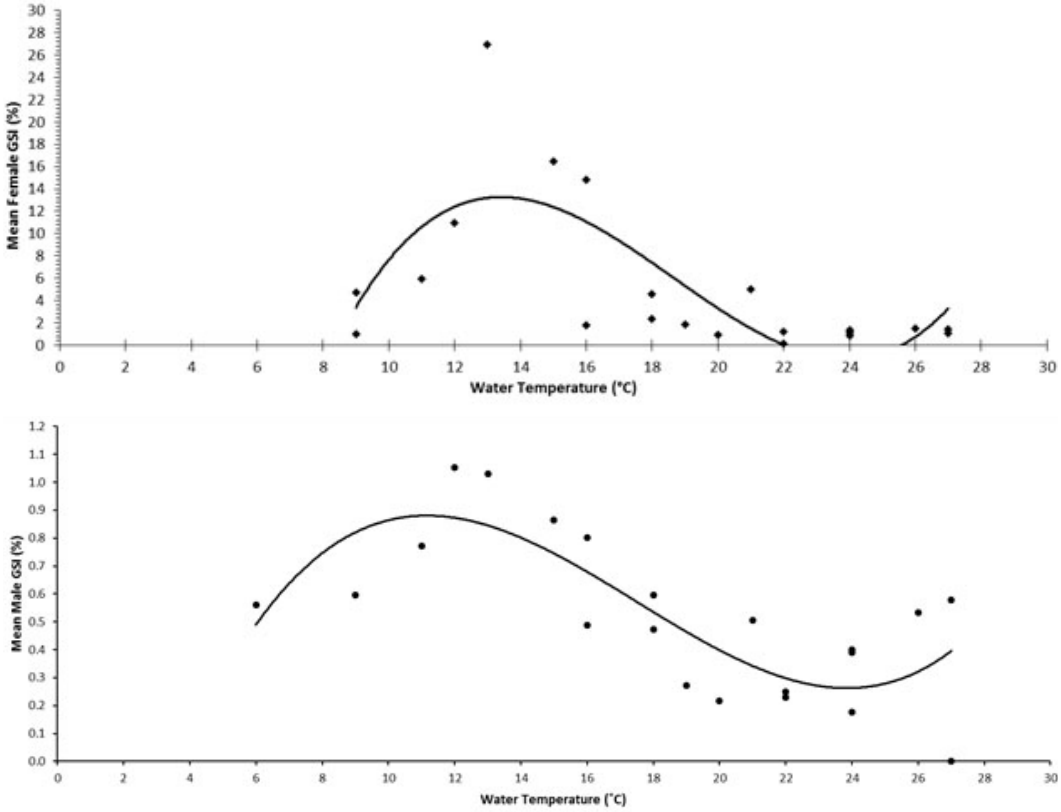

Figure 10: Third-order polynomial trend lines comparing water temperature at times of sampling to GSI values of specimens. Top graph Females, bottom graph Males.

Sex bias and size structure

The total number of fish collected from August 2011 to July 2013 was 423, including: 170 females, 189 males, and 64 juveniles (Table 4). Juveniles were present in June 2012 – October 2012, July 2013, and a single occurrence in January 2013. The mean SL in females was 84.6 mm (SE = 1.36) and of males 84.4 mm (SE = 0.801). No significant difference was found between the number of females and males caught over the two year sampling period, $\chi^2_{(1, 359)} = 1.001$, $p = 0.316$.

Year/Month	Females	Males	Juveniles	Total
2011				
August	11	14	0	25
September	3	4	0	7
October	4	3	0	7
November	3	1	0	4
2012				
February	8	5	0	13
March	9	7	0	16
April	8	5	0	13
May	4	7	0	11
June	2	0	18	20
July	9	8	14	31
August	13	11	1	25
September	18	14	1	33
October	14	21	3	38
November	23	18	0	41
December	0	1	0	1
2013				
January	1	0	1	2
February	4	3	0	7
March	7	6	0	13
April	11	25	0	36
May	5	14	0	19
June	6	14	5	25
July	7	8	21	36

Table 4: Total numbers of males, females, and juveniles collected by month from August, 2011, through July, 2013. No individuals were collected in December, 2011, or January, 2012, due to high flow river conditions.

DISCUSSION

Successful reproduction for *N. photogenis* in north Alabama in late winter and early spring requires seasonal pulses in river discharge with river temperatures in the range 12º – 17 ºC (Figure 10). River flow is known to be important in reproduction for broadcast spawners, probably including *N. photogenis*, and can influence species abundance as well (Durham and Wilde, 2009). But too much stream flashiness, along with low dissolved oxygen, and water temperature fluctuations during times of reproduction, could lead to the death of free embryos and population declines for broadcast spawners. Stream fishes select for microhabitat features such as flow, depth, and substrate (Mueller and Pyron, 2009). Observations over two years in this study showed associations between daily mean discharge and months of peak GSI (February, March, April) for *N. photogenis* (Figure 9). Mean GSI peaked in March of both 2012 and 2013

when median flow was approximately 600 cfs (Figure 9). Median discharge ranged between 500–650 cfs from February to April before beginning to rapidly decline in May. If other river conditions, primarily temperature, are right for ovarian maturation, pulses in river discharge appear to be the necessary trigger for stimulating the final full maturation of ovaries in *N. photogenis*. Variability in river discharge in late winter and early spring can be expected from year to year, especially in years that coincide with El Niño and La Niña or other climate oscillations (Mazouz et al., 2012; Ingram et al., 2013; USGS, 2015). This suggests that the onset and duration of spawning by *N. photogenis* will vary interannually.

Sex and Size

As expected of non-sexually dimorphic fish, there was no significant difference in the number of males to females caught. Juveniles were present June–October and found predominately towards the shallow banks of the river or in side channels receiving minor flow and near aquatic vegetation.

Reproductive Schedule

Spawning takes place in spring and summer months for most North American cyprinids (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 *N. photogenis*, oocyte size is not evenly distributed at the peak of reproduction. 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 (Heins and Rabito, 1986). As expected, our study identified late winter and early spring as the months of reproduction with GSI values peaking in March both years. Since *N. photogenis* is a multiple spawner, multiple stages of oocyte development were found to occur throughout the three month reproductive season (Warren and Burr, 2014). Spawning events (Figure 10) were identified in 2012 and 2013 by drops in GSI and oocyte diameter. Only one spawning event was identified for 2012, likely due to the fact that the eight female fish caught in April of that year had an average GSI of < 3%. If sample size was increased it is possible that females with a higher GSI would eventually have been caught. However, sizes for these sampled females ranged from 88–111 mm, indicating they were large enough to be reproductively competent even though low GSI values indicated they were not. It is possible that rising water temperatures in 2012 may have influenced early termination of the breeding season. For 2013 two spawning events were identified both by drops in GSI and oocyte diameter

between sampling times. It is possible that oocytes are shed in small amounts asynchronously throughout the reproductive season. However, the large synchronous events appear to occur during times of steady significant discharge increases, after peaks of discharge > 3000 cfs.

Baldwin (1983) noted that the reproductive months are May and June for *N. photogenis* in Ontario, Canada, with the first young of the year caught in June as well. Baldwin also noted that ovary size began to increase in August. Due to the colder temperatures at higher latitude, this northern population may have a shorter spawning season than conspecifics in Alabama, consistent with the predictions of Gotelli and Pyron (1991).

Ovarian Development, Oocyte Diameters, Fecundity and Clutch Size

Only fish containing ripe oocytes were used in evaluation of oocyte diameter (Heins, 1995). Individuals may be able to adjust the size of their oocytes due to phenotypic plasticity (Duarte and Alcaraz, 1989). Variation in oocyte size is more pronounced in species producing demersal eggs than those with pelagic eggs such as *N. photogenis* (Duarte and Alcaraz, 1989). The relationship between offspring and environment is likely influenced by maternal phenotype. Ripe oocytes of *N. photogenis* were not found to vary in size during the years of collection, which was expected due to the pelagic nature of their offspring. This species is thought to be a multiple synchronous spawner along with others in the subgenus *Notropis,* however the practice has not been witnessed in the field or confirmed using histological methods (Winemiller and Rose, 1992). Complete clutch sizes were determined for the 17 females with advanced stage maturation ovaries. The biggest individual did not have the greatest clutch size or GSI just as the smallest individual did not have the lowest clutch size or GSI (Table 1). Total fecundity varied with the smallest fecund female carrying over twice the amount of oocytes as the largest fecund female. This demonstrates that body length alone does not determine fecundity. Variation in fecundity-length relationship is likely tied to the variation in spawning pattern, synchronous or asynchronous, observed in other North American stream cyprinids such as *N. buccula* (Smalleye Shiner), *Hybognathus placitus* (Plains Minnow), and *N. oxyrhynchus* (Sharpnose Minnow) by Durham and Wilde (2014), is widespread among broadcast spawning prairie stream and river fishes. The prairie species studied by Durham and Wilde (2014) live in streams with different geomorphology and hydrology than the Flint River but appear to respond in a similar fashion to fluctuating stream flow. While the current study focuses on population scale data, histological analysis to confirm asynchronous spawning of *N. photogenis* at the individual level would be beneficial.

Other cyprinid species recently studied in the Flint River include *Erimystax insignis* (Blotched Chub) (Stallsmith et al., 2015) and *Hybopsis amblops* (Bigeye Chub) (Crissy Tarver, unpublished manuscript). *Erimystax insignis* showed peak ovarian development in March and April, and peak female GSI from March through May. The species was also characterized by possessing relatively small mature oocytes in large numbers compared to other published reports about stream cyprinids in the southeastern United States, suggesting it is also a pelagophil (Stallsmith et al., 2015). *Hybopsis amblops* showed peak ovarian development later in the season, April and May, as measured by female GSI, ovarian maturation, and largest numbers of late stage oocytes, but with overall fewer and larger oocytes compared to regional stream cyprinids (Crissy Tarver, unpublished data). These data support an interpretation that *N. photogenis* and *E. insignis* are pelagophils, spawning earlier in the season when river flow is typically high compared to a likely non-pelagophil like *H. amblops.* Such high flow beneficially affects pelogophil larval development by delivering the larvae to suitable habitat for maturation within a time-frame of six to seven days (Dudley and Platania, 2007).

These findings support what is widely known among aquatic ecologists and resource managers, that free-flowing rivers such as the Flint are necessary to the successful reproduction of many resident species. Other streams in north Alabama where *N. photogenis* has been spottily observed such as the upper Elk River and Shoal Creek are also free-flowing, subject to seasonal flow pulses. Without these seasonal pulses *N. photogenis* and other likely pelagophils such as *E. insignis* are likely to disappear from such streams.

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