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Reproductive Schedule of the Largescale Stoneroller (*Campostoma oligolepis*) in North Alabama, USA

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Abstract: Understanding the reproductive patterns and strategies of a species is an important step in establishing the species' life history. *Campostoma oligolepis*, the Largescale Stoneroller, is a species that has received little attention in the 90 years since it was first identified, and the work that has been done has been localized in the American Midwest. Collections of *C. oligolepis* were made monthly from the Flint River in Madison County in northern Alabama, USA, from March, 2014, to September, 2015. A total of 768 fish were collected over the collection period including 492 adults, 268 females and 224 males. We found strong evidence that the peak spawning time for *C. oligolepis* in the Flint River is March and April. Ovarian maturation, gonadosomatic index for both sexes, and monthly clutch size all support this conclusion. Two unexpected features were found. The first is how few females of mature size were found to carry either oocytes or a clutch except in the peak observed reproductive month of April, 2014. The second unusual feature is the prevalence of asymmetric ovaries, with the left the larger if a difference exists. *Campostoma oligolepis* may have unusually strong inter-annual responses to abiotic factors such as water temperature and river discharge.

Keywords: cyprinidae; life history; freshwater ecology; oocyte; ovary.

1. Introduction

Understanding the reproductive patterns and strategies of a species is an important step in establishing the species' life history. *Campostoma oligolepis*, the Largescale Stoneroller, is a species that has received little attention in the 90 years since it was first identified, and the work that has been done has been localized in the American Midwest. Very little is known about this species' reproductive season or strategies, and what information that can be found on the topic is conflicting and often inferred from what is known about other species within the genus *Campostoma*. This research takes a closer look at *C. oligolepis* in the Southeastern United States to establish a clear understanding of its reproductive timing.

Campostoma oligolepis inhabits clear running rivers and streams with substrates of cobble and gravel. Adults prefer fast riffles or moderately deep riffle runs and have a very low silt tolerance, while juveniles frequently favor slower currents over sand and silt [1]. *Campostoma* itself is Latin for "curved mouth" stemming from their prominently curved jaws that allow them to more easily scrape algae and detritus material off the benthic substrate with their cartilaginous lower jaws [2, 3].

There is little if any consensus on the reproductive schedule of *C. oligolepis*. They are reported to spawn from early April to June by some sources [1, 2], and March through April by others [4], but there are no peer-reviewed, published data to support that claim. In fact, the only published paper focused on *C. oligolepis* reproduction found them to be reproductively active from January through May contrary to all other claims [5]. However, their study was intended to identify reproductive differences in *C. oligolepis* between urban and rural streams, and it neglected to collect specimens during 4 months of the calendar year based on the claim in [2] that they are only reproductively active from April to June. It does, however, further emphasize the general disagreement on when *C. oligolepis* is reproductively active.

What we do know about *C. oligolepis* reproduction is largely based on what is known to be true of all *Campostoma* species. During breeding season, males dredge out spawning pits in shallow water with gravel substrate. This is done by shuffling stones with their mouths or heads, earning them the name "stonerollers." A small numbers of males grow larger in size and more colorful and monopolize ova fertilization. These large males guard their spawning pits which may be as large as 30 cm deep. Growing larger than other males and guarding spawning pits requires a large investment of energy but is rewarded with greater reproductive success. Other males conserve their energy by not growing as large and through sneaky mating behavior in which smaller males attempt to fertilize ova in another male's guarded territory [6]. Multiple females may visit a pit and spawn with the male(s) guarding it, and males may create several spawning pits in a breeding season.

Like most teleost species, once spawning is complete, the nest is left unattended and no parental care is provided to the fry upon hatching. *Campostoma anomalum* has been known to compete for reproductive sites with rainbow trout and creek chub, often destroying the other species' nests in order to make their own [6]. The spawning area created by *Campostoma* males, with clean, upturned gravel, creates an ideal area for many other species to spawn. In this way, *Campostoma* contributes to the maintenance of many other fishes in its ecosystem [2, 3, 7].

The closely related *C. anomalum* has a peak reproductive season from early April to mid-June with water temperatures from 12 to 14 ˚C. Females deposit 200–2500 eggs during breeding season [2]. A study in Pennsylvania found that mature *C.a. pullum* eggs measured around 2.0 mm, and hatching occurred approximately 69–72 hours after fertilization with newly emergent larvae measuring an average of 5.7 mm in standard length [8]. Young are estimated to reach sexual maturity in their first year while reaching lengths of 35 to 65 mm [2].

Campostoma oligolepis is abundant where found and presumably important to riverine community ecology. Our study population is from the Flint River in the Tennessee River drainage of north Alabama. Establishing reproductive timing and production is an essential step in understanding the life history of a species. In particular we are interested in establishing the months with peak gonadal maturation, clutch size in females, and the size of oocytes at different developmental stages. Does the reproductive biology of *C. oligolepis* match what is known of its congener *C. anomalum?* And do peak reproductive months match what has been suggested in books reviewing the fishes of a given state? The authors in [2] suggest April through June for Tennessee, while others in [9] say that tuberculate males are seen mostly in April in Alabama. River temperature likely plays a role in determining the onset of spawning. Average river discharge was also calculated for collection days based on a U.S. Geological Survey monitoring station downstream from the collection site. Early in this research we noticed what seemed to be an unusual feature for a North American cyprinid, in that many females had markedly larger left compared to right ovaries. So we also collected data to establish whether or not such ovarian size difference is a consistent feature in this species.

2. Results

In some months two collections were made if a first collection yielded too few fish. A total of 768 fish were collected over the collection period of 19 months including 492 adults, 268 females and 224 males. Water temperature varied from 4.3 °C in December to 29.9 °C in June. River discharge varied from 110 ft^3/sec in September to 1060 ft^3/sec in December (Table 1). Flood events can cause much higher river discharge, but experience has shown that safe collection can only be done below about 1000 ft^3/sec.

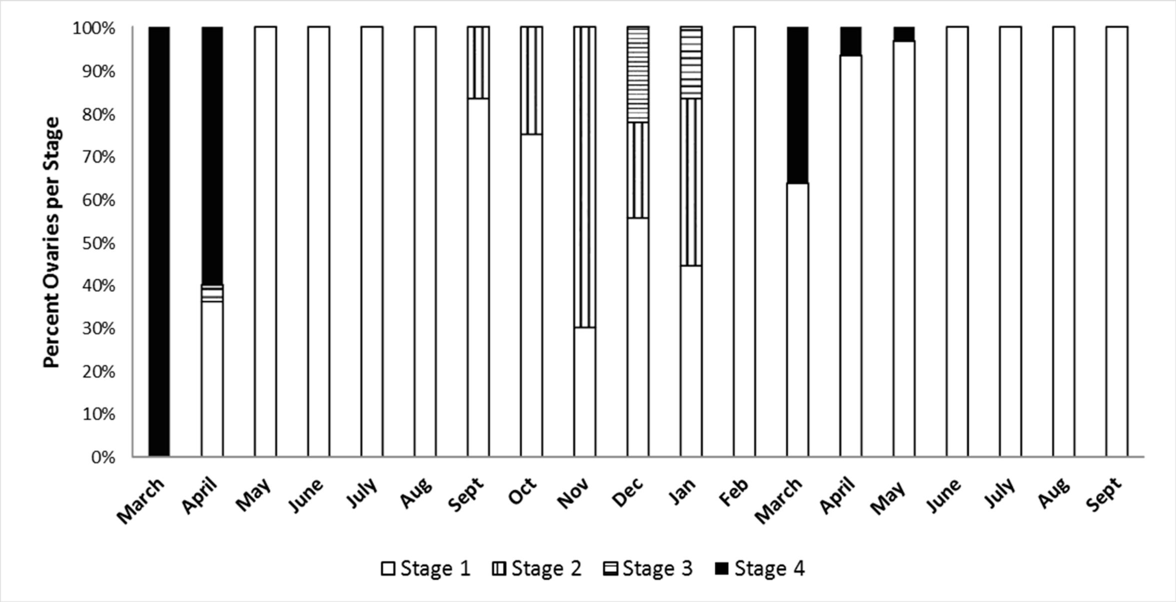
Table 1 Water temperature and river discharge in cubic feet per second on fish collection dates. Water temperature was measured during fish collections. River discharge is a daily average of hourly measurements from a U.S. Geological Survey monitoring station downstream of collection site, and is reported as ft$^{3/s}$ because that is how the U.S. Geological Survey reports river discharge.

Date	Temp (˚C)	Water Discharge (ft3/s)
2014		
March 11	15.8	465
April13	20.7	589
April 18	15.4	632
May 9	21.1	409
June 20	29.9	334
July 3	23.9	258
August 12	23.9	178
September 19	24.1	120
September 27	21.3	110
October 12	21.5	404
October 26	18.0	199
November 8	14.8	241
December 14	4.3	298
December 31	8.7	1060
2015		
January 17	9.6	854
February 7	10.6	486
March 21	15.5	917
April 24	17.8	844
May 4	19.2	427
May 10	23.4	316
June 19	28.3	183
July 17	27.0	190
August 16	27.6	142
September 27	21.7	135

Monthly ovarian maturation is shown in Figure 1. Few stage 4 ovaries were found, almost exclusively in March and April of both years. Almost all of the stage 3 ovaries were found in

December and January, but no stage 3 ovaries were found in February which was restricted to stage 1 ovaries. Stage 2 ovaries were found from September through January. Ovaries examined from May through September were nearly exclusively stage 1.

Figure 1 Monthly stages of ovarian maturation.



GSI as a measure of reproductive status of both sexes indicates March and April in both years as peak reproductive months, but peak values did not always co-occur for both sexes (Figure 2A for females, and 2B for males). The peak GSI for both sexes in 2014 is clearly in April. For 2015 peak female GSI is in March, with a sharp drop off in April, and male GSI is elevated in both March and April. An unusual finding is that both sexes show a moderate increase in GSI values for November, December and January with very low values in February before rising in March.

Figure 2A. Average monthly GSI for females. Error bars are one standard error.

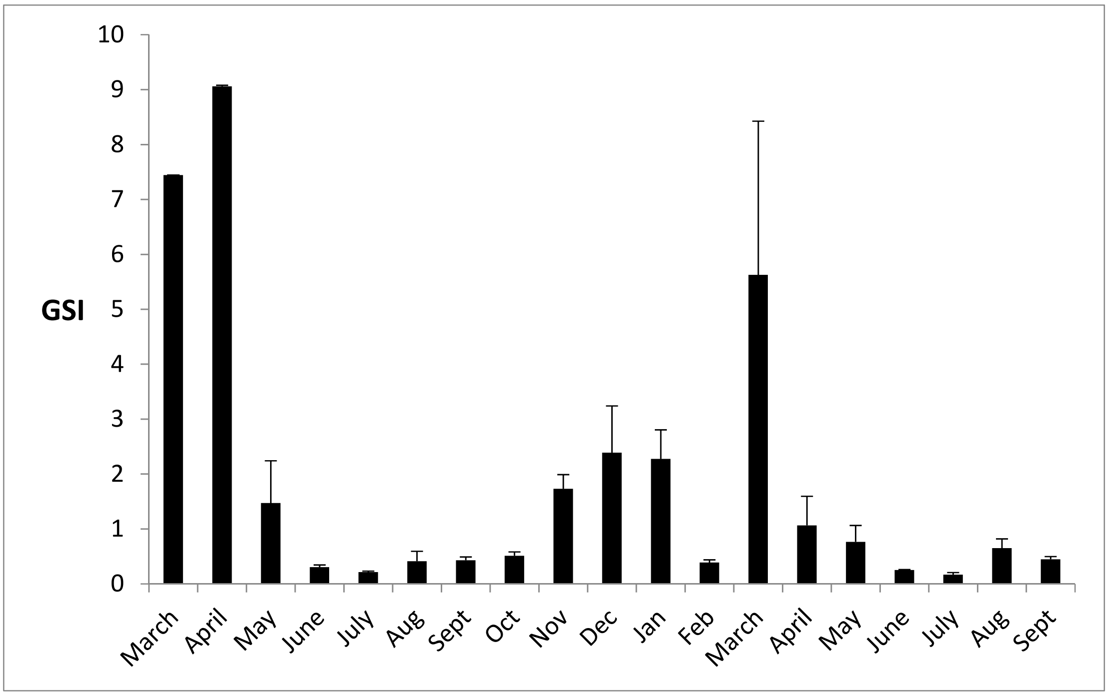
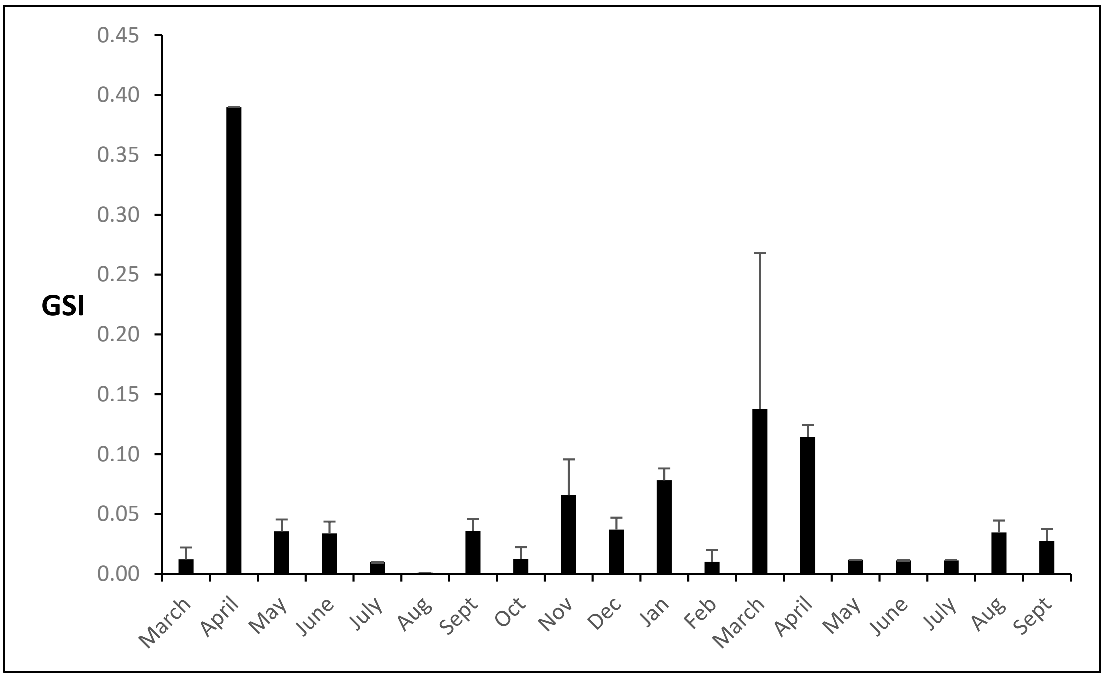


Figure 2B. Average monthly GSI for males. Error bars are one standard error.



Monthly averages of total oocytes show the same patterns as GSI (Table 2). April, 2014, has by far the largest observed average number of total oocytes at 13,733 along with largest GSI. But the block of three winter months with elevated GSI, November through January, averaged between 6,380 and 7,432 total oocytes. No oocytes were found in the five females examined in February, followed by a GSI spike in March with an average of 2,293 oocytes per females and both values dropping steeply in April. Also unusual is the finding that in months with elevated oocyte and GSI values, some females were found with no oocytes. Five of 25 females examined in April, 2014, had no oocytes, and seven of 11 females examined in March, 2015, had no oocytes. The presence of females with no oocytes is also found in November through January. The presence of significant numbers of stage III and IV oocytes, those ready or nearly ready for deposition, is restricted to March through May of both years. In the months of November through January with elevated oocyte counts the strong majority of oocytes were stage I.

Table 2 Monthly averages of oocytes per female by both stage and total of all stages are shown. The total number of females examined each monthly is reported, along with how many of those females were found to be carrying stage I or later oocytes.

	Stages				**Total**	**Females with**	
Month	**I**	**II**	**III**	**IV**	**Oocytes**	**oocytes**	**Total Females**
2014							
March	0	519	525	15	1059	1	1
April	435	2004	10691	603	13733	20	25
May	14	98	147	14	273	4	8
June	0	0	0	0	0	0	11
July	0	0	0	0	0	0	5

August	0	0	0	0	0	0	16
September	14	0	0	0	14	1	6
October	161	0	0	0	161	1	4
November	5019	1522	0	0	6541	7	10
December	3965	2398	17	0	6380	4	9
2015							
January	4276	3149	7	0	7432	8	17
February	0	0	0	0	0	0	5
March	331	706	1186	170	2393	4	11
April	4	15	159	23	201	1	15
May	70	131	431	19	650	10	30
June	0	0	0	0	0	0	49
July	0	0	0	0	0	0	9
August	0	0	0	0	0	0	22
September	0	0	0	0	0	0	14

Monthly clutch sizes are shown in Table 3. Almost all females carrying a clutch were found in March, April or May, with the exception of a single fish in December. March and April of both years are the peak clutch months with monthly averages of 182–594 and total counts of 6–1359. Only in April, 2014, did a majority of examined females carry a clutch.

Table 3 Monthly clutch sizes, the number of stage III plus stage IV oocytes found in a stage 3 or 4 ovary. Number of adult females found carrying a clutch is in parentheses in the Range column.

Month	**Monthly Average**	**Range**	**Total Adult Females**
2014			
March	540	540 (1)	1
April	594	22–1359 (19)	25
May	40	6–60 (4)	8
December	17	17 (1)	9
2015			
March	339	16–608 (4)	12
April	182	182 (1)	15
May	68	1—50 (9)	30

Average oocyte diameters were in general largest in March and April both years (Table 4). The mean diameters from both years combined ranged from 0.57 mm for stage 1 to 1.67 mm for stage 4. There was very little size overlap between different stages.

Table 4 Oocyte Diameters (mm) by stage. Three oocytes from all stages present in a female were measured and averaged, and those averages from all females in a month were used to compute averages for each stage. A notation of n.a. indicates a stage was not present in that month.

Month	**Stage I**	**Stage II**	**Stage III**	**Stage IV**
2014				
March	n.a.	0.91	1.42	1.78
April	0.70	1.03	1.68	1.88
May	0.61	1.01	1.37	1.65
September	0.41	n.a.	n.a.	n.a.
October	0.40	n.a.	n.a.	n.a.
November	0.51	0.70	n.a.	n.a.
December	0.61	0.92	1.26	n.a.
2015				
January	0.57	0.77	1.04	n.a.
March	0.58	0.91	1.40	1.48
April	0.66	1.15	1.61	1.71
May	0.68	1.04	1.39	1.52
Mean	0.57	0.94	1.40	1.67

A paired t-test was used to test the hypothesis that the left ovaries of examined fish contained significantly more oocytes than the right ovaries (Table 5). This test yielded p = 0.048, supporting the hypothesis that the left ovary contains more oocytes.

Table 5 Monthly average of total oocytes in the left and right ovaries. A paired t-test was used to test for differences between total oocytes in the left and right ovaries over nine months when oocytes were present. This test reported $p = 0.048$, supporting a finding of significantly more oocytes in the left ovary.

Month	**Left**	**Right**
2014		
March	777	282
April	4313	3359
October	63	98
November	3851	2690
December	3078	3302
2015		
January	2981	2335
March	1020	772
April	106	95
May	337	264

3. Discussion

We found strong evidence that the peak spawning time for *C. oligolepis* in the Flint River is March and April, with observed river temperatures of 15.4–20.7 °C and river discharge between 465 and 917 ft^3/s. Ovarian maturation, GSI for both sexes, and monthly clutch size all support this conclusion. This also supports statements based on field observations of physical condition [4, 9].

Two unusual and unexpected features were found in our population of *C. oligolepis*. The first is how few females of mature size were found to carry either oocytes or a clutch except for the peak observed reproductive month of April, 2014. This is markedly different from other cyprinids examined from the Flint River such as *Erimystax insignis* [10], *Notropis photogenis* [11], or *Lythrurus fasciolaris* [12]. We offer two possible explanations: adult females simply do not all enter oogenesis in a given season, or alternatively that they can be spawned out early for a season without further oogenesis, The first explanation is supported since we found that about half of all females in the November – January period carried no oocytes at a time when all would be expected to carry maturing early stage oocytes for the spring spawning season. The second explanation seems unlikely since cyprinids in the southeastern region of the United States are iteroparous within a spawning season.

The second unusual feature is asymmetric ovaries, with the left the larger if a difference exists. We are aware of few similar observations in teleost fishes. For example, Kobelkowsky [13] examined 90 Mexican teleost species and found only one, the anchovy *Anchoa mitchilli*, with the right ovary smaller while the larger left ovary grows below the intestines similar to *C. oligolepis*. We found that the left ovary in *C. oligolepis* wrapped around the large intestinal mass typical of the species often in several loops. We do not know why this may be, and why is it invariably the left ovary that is expanded, but offer the suggestion that it may be easier to pack one very large ovary into the crowded abdomen rather than two large ovaries. To our knowledge this has not been observed in other *Campostoma* species.

The reproductive effort and timing of *Campostoma anomalum* can be more variable than simply large males building and guarding pits over which they spawn. The species may show facultative reproductive behavior at nests constructed by *Nocomis leptocephalus*, the Bluehead Chub [14]. Other stream minnow species are obligate communal nest spawners but *C. anomalum* may only spawn over *N. leptocephalus* nests in years in which stream temperatures are slow to rise. In southwestern Virginia, *C. anomalum* can begin spawning at 10.8 °C while *N. leptocephalus* spawns at 13–15 °C. *Campostoma anomalum* were observed to spawn over their own nests several weeks ahead of the construction of *N. leptocephalus* nests in a stream [14]. But *C. anomalum* males were observed to dig deep trenches in *N. leptocephalus* nests with no evidence of spawning in them that year. Whether or not this is typical the authors could not really say. Whether *C. oligolepis* has any tendency toward facultative reproductive behavior is unknown.

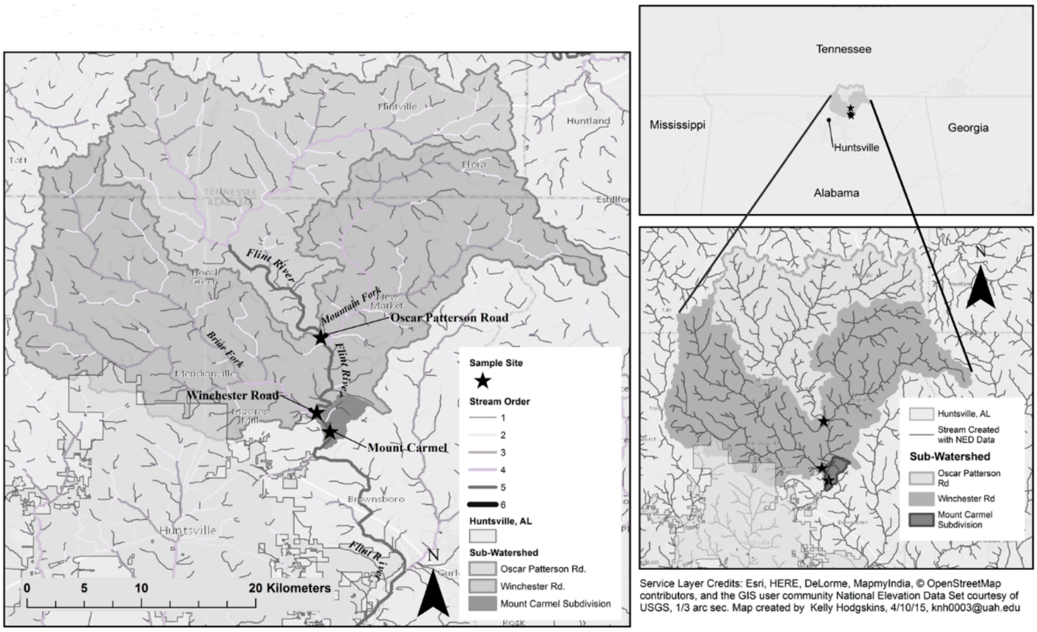
Campostoma oligolepis may have unusually strong inter-annual responses to abiotic factors such as water temperature and river discharge. Our data set collected over two spawning seasons and 19 months hints at such pronounced variability, more than has been observed with sympatric cyprinids in the Flint River [11, 12]. This species' nest-building strategy may make *C. oligolepis* more sensitive to inter-annual abiotic variation.

4. Materials and Methods

Collection sites and fish capture

Collections of *C. oligolepis* were made monthly from the Flint River in Madison County in northern Alabama from March, 2014, to September, 2015 (Figure 3). 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 [15], and its major branches are a total of 562 km long. 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. 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 [11]. The collection site was the Oscar Patterson Road crossing of the river (34° 52″ 50′ N, 86° 28″ 50′ 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 *C. oligolepis* habitat; medium-sized, clear-water streams with rock, pebble and gravel substrates and moderate water flow [9].

Figure 3 A map of the Flint River drainage indicating the location of the Oscar Patterson Road collection site.



Service Layer Credits: Esri, HERE, DeLorme, MapmyIndia, © OpenStreetMap contributors, and the GIS user community National Elevation Data Set courtesy of USGS, 1/3 arc sec. Map created by Kelly Hodgskins, 4/10/15, knh0003@uah.edu

Median daily river discharge data for all collection dates were obtained from USGS river gauge #03575100 located downstream in Brownsboro, AL [16]. Water temperature measurements were taken during each collection as an indicator of the abiotic environment. Fish were collected using a seine net measuring 3.5 m long, 1.2 m wide with a 1.3 mm mesh. The net was placed in moderate depth (0.5 – 1. 0 m) riffles over a gravel and cobble substrate. All adult *C. oligolepis* (> 55 mm) were collected indiscriminately of sex along with juveniles to be used as a reference of size variation throughout the year. Excess obvious juveniles in some collections were released on site. The adult threshold of 55 mm was based on early observation in the project of a lack of gonadal maturation in specimens measuring less than 55 mm. As fish were not measured on site, juvenile status was based on estimation, and any individuals apparently near 55 mm were kept. All collected specimens were euthanized on site by adding 2–3 ml of a 9:1 ethanol/clove oil solution to about 500 ml of water in the collection bucket, and then transferred into a 10% phosphate buffered formalin solution for fixation until they could be dissected and analyzed.

Laboratory analysis

Standard length (SL) was measured with digital calipers to the nearest 0.01 mm. Gross body mass was obtained to the nearest 0.0001 grams using an Explorer OHAUS digital balance after excess fluid was blotted from the fish's body. The sex of each fish was determined by excision and examination of gonadal tissue using an Olympus SZX7 or a Motic K series dissecting microscope. After excess surface fluid was blotted away from the gonadal tissue, gonadal mass was obtained to the nearest 0.0001 g. Gonadosomatic Index (GSI) was calculated as: GSI = (body mass – gonadal mass)/body mass X 100 [10, 11, 17].

Images of intact gonads and oocytes were captured using an Olympus SZX7 dissecting microscope with an Olympus DP72 camera. Ovaries that were too large to be captured in a single frame were also photographed using a Sony Cyber-shot 20.1-megapixel digital camera. The images were later analyzed for maturation status and number (for oocytes) using the CellSens Standard software (Ver. 1.5) that comes with this camera. Each microscope image was captured at 8.4X and saved as a .tiff file.

Ovarian maturation was assessed for each female using a modification of the scheme described in [18]. Based on macroscopic development, ovaries were divided into five stages (Fig. 2). Immature (stage I) ovaries are small in size, usually opaque, and contain only latent oocytes. Maturing (stage II) ovaries are larger, inhabiting a larger portion of the abdominal cavity. Maturing ovaries contain various sizes of white and cream colored oocytes. Advanced maturation (stage III) ovaries are bulkier and densely packed with oocytes. The oocytes are yellow to orange, and various sizes of vitellogenic oocytes are visible in between oocytes that are ready to be released during spawning. Ripe (stage IV) ovaries are partially ovulated and oocytes are released when squeezing the fish's sides. In stage IV the ovary has obtained maximal development, but vitellogenic oocytes of several sizes are present in between the mature oocytes due to multiple spawning. Spawned and recovering (stage V) ovaries are still relatively large and flaccid with remaining empty spaces, but contain different sizes of developing vitellogenic oocytes. This stage can occur in between spawning cycles, and is also indicative of the end of the spawning season.

Both ovaries from each female were teased apart using 21-gauge hypodermic needles to liberate developing oocytes from the ovarian tissue. During dissection, the left ovaries of gravid females were often visibly larger than the right. Oocytes liberated from ovaries that remained intact after dissection and storage were labeled as being either from the left (L) or right (R) ovary, and eggs that had fallen loose of the ovary during either removal or storage were labeled as unknown (U). Oocytes were arranged into a single layer on a Syracuse watch glass to be photographed. When the number of oocytes exceeded one frame, multiple frames were taken. Digital images were used to categorize oocytes into stages of maturation using the schematic of [18]. Latent oocytes were not counted in this project. Early maturing (stage 1) oocytes are previtellogenic and are distinguished by their small size which is half the diameter of a ripe oocyte. Late maturing (stage 2) oocytes are in early vitellogenesis and contain small yolk granules. The diameter size is larger, and a nuclear envelope can be seen. Mature (stage 3) oocytes are in late vitellogenesis, yellow in color and filled with yolk globules. The vitelline membrane is obviously divided from the yolk. Ripe (stage 4) oocytes have a larger diameter than all other oocytes and are yellow to dark yellow-brown in color with vitelline membranes that are completely separated from the yolk mass. 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 is reported as clutch size [10, 11, 19, 20]. This represents the number of mature oocytes nearly or immediately ready for spawning.

All oocytes (excluding latent oocytes) were counted by stages and the total number was calculated for each female. Oocyte counts were performed using EggHelper, a custom program developed in Microsoft Visual Studio 2013, and confirmed using CellSens software [21]. The diameters of three oocytes per developmental stage per female were measured, and monthly averages for each stage were calculated for each female.

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