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Background. The purpose of this study was to establish a reproductive schedule and examine reproductive traits that shape fecundity of the Bigeye Chub, *Hybopsis amblops* Cyprinidae, in the Flint River system of north Alabama.

Methods. Life history traits associated with reproduction, growth, and maturation were assessed. Fish collections were made monthly from August, 2013, through July, 2014.

Results. The Bigeye Chub in Alabama primarily spawns in April and May as indicated by gonadosomatic index (GSI), ovarian condition and clutch size. Average GSI values began to rise in February, peaked in April and May at over 13% for females and 1.6% for males, and showed a steep decline from May to June for both sexes. Average clutch size was highest in April at 812. Diameter of the most mature oocyte stage averaged 0.74 mm, relatively small compared to other cyprinids found in the Flint River.

Discussion. The Bigeye Chub's relatively large clutch size as a measure of fecundity places the species intermediate between opportunistic and periodic in the trilateral life history scheme of Winemiller and Rose. The species is apparently responding to a flow regime with a defined seasonality as well as predictability of flow and resources.

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Methods. Traits associated with reproduction, growth, and maturation were assessed. Specimens were collected and examined monthly from the Flint River in northern Alabama from May, 2013 until July, 2024.

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Discussion. The Bigeye Chub's relatively large clutch size as a measure of fecundity places the species intermediate between opportunistic and periodic in the trilateral life history scheme of Winemiller and Rose. Results of this study indicate Bigeye Chubs found in the Flint River in Alabama primarily spawn in April and May, which is slightly earlier than the late spring and early summer previously predicted. The collection of this information was important for future comparisons showing this population's response to environmental changes, sympatric and allopatric population comparisons, and any conservation effort based on scientific data.

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Introduction.

Hybopsis amblops (Rafinesque), the Bigeye Chub, is a leucisid minnow found in the Lake Ontario and Lake Erie drainages, Ohio River basin, and south to the Tennessee River drainage in northern Alabama (NatureServe, 2014). They are currently thought to be extirpated in the Missouri river drainage, and rarely found in segments of the Illinois River drainage. Bigeye Chubs also appear to be extirpated in the Kaskaskia and Wabash River drainages and branches of the Ohio River (Warren and Burr, 1988). This species is frequently collected over hard sand or gravel substrates in areas of low current, and they have a low tolerance to siltation. Their decline in distribution range has been linked to the degradation and pollution of aquatic ecosystems (Warren and Burr, 1988).

There are limited studies and a general lack of information on Bigeye Chubs. They have been collected over hard sand and gravel substrates in areas of low current. According to literature, members of this species attain a maximum standard length of 80 mm and anecdotally spawn during the late spring and early summer (Boschung and Mayden, 2004). They are considered to be lithophilic spawners, utilizing gravel and mineral substrates instead of simply releasing eggs into the sediment (Frimpong and Angermeier, 2013). Data concerning female fecundity could not be found in the literature but Frimpong and Angermeier (2013) suggest a value of 1,000 eggs for maximum fecundity. A diminishing number of fish and insufficient data concerning their reproductive biology inspired this study. While data shown here are limited to a 15-month study period, it provides vital reproductive information for Bigeye Chubs. It makes available knowledge on the condition of the existing population and presents a point of reference for management of this species.

The purpose of this study was to establish a reproductive schedule and examine reproductive traits that shape fecundity of the Bigeye Chub in the Flint River system of north Alabama, at the southern edge of its range. Life history traits associated with reproduction, growth, and maturation were assessed. Reproductive traits of North American stream leucisids including timing, oocyte size and clutch size are known to be affected by water temperature and the volume of stream flow, so those data were collected for each monthly fish collection (Olden and Kennard, 2010; Bennett et al., 2016). We also wished to compare this species to sympatric leucisids in the Flint River which have been similarly studied including *Erimystax insignis* (Hubbs and Crowe)*,* the Blotched Chub (Stallsmith et al., 2015), *Notropis photogenis* (Cope), the Silver Shiner (Hodgskins et al., 2016), and *Lythrurus fasciolaris* (Gilbert), the Scarlet Shiner (Stallsmith et al., 2018).

Materials & Methods

Study site and sampling

The Flint River system includes approximately 147,151 hectares in Madison County, Alabama, and Lincoln County, Tennessee (Abidi et al., 2009). The Flint River is free flowing with varying levels of discharge (Fig. 1). Mean monthly discharge for each month of collection was obtained from the U.S. Geological Survey database (USGS, 2014). Clear to moderately turbid water runs over exposed Tuscumbia limestone and chert. The substrate consists of boulders, large cobble, small cobble, sand, silt, and mixtures of each. Aquatic foliage grows freely on the bars and banks, with the river surrounded by mostly agricultural land.

The Oscar Patterson Road bridge access (34˚48’24” N, 86˚28’21” W) on property owned by the Land Trust of North Alabama was the entry point used in this study (Fig. 2). Collections were made monthly from May, 2013, through July, 2014, overlapping primary spawning months. Female specimens were not available for examination in January, 2014. Only one female specimen was available for examination in July, 2013, and February, 2014. All fish were collected from a 300 m length of the Flint River. Fish were netted using a seine net measuring 3.5 m length, 1.2 m depth, and a 3 mm mesh. A cast net was with a radius of 2.5 m and a 6 mm mesh was used occasionally. All fish were euthanized on site using a 1:10 clove oil: 95% ethanol solution diluted with 350 ml river water. Samples were then placed in commercial grade 10% phosphate buffered formalin for tissue fixation and storage. Water temperature was taken during each collection with an alcohol thermometer. The average rate of river discharge for each month of collection was obtained from the U.S. Geological Survey databank (USGS, 2014).

Laboratory analysis

Standard length (SL) was measured with digital calipers to the nearest 0.01 mm. Gross body mass was obtained to the nearest 0.01 g with 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 under an Olympus SZX7 dissecting microscope. After excess surface fluid was blotted away from the gonadal tissue, gonadal mass was obtained to the nearest 0.001 g. Gonadosomatic Index (GSI) was calculated as: GSI = (body mass – gonadal mass)/body mass X 100. Images of intact gonads and oocytes were captured under an Olympus SZX7 dissecting microscope with an Olympus DP72 camera. The images were later analyzed for maturation status and number using the CellSens Standard software (Ver. 1.5) that comes with this camera. Each image was captured at 8.4X (1.6X x 4.0X) and saved as a .tiff file.

Ovarian maturation was assessed for each female using a modification of the scheme described by Núñez and Duponchelle (2009). Based on macroscopic development, ovaries were divided into five stages. 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 sides of the fish. 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 (Fig. 3). This stage can occur in between spawning cycles, and 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. All 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 Núñez and Duponchelle (2009). Latent oocytes were not counted in this project. Early maturing (stage I) oocytes are previtellogenic and are distinguished by their small size which is half the diameter of a ripe oocyte. Late maturing (stage II) oocytes are in early vitellogenesis and contain small yolk granules. The diameter size is larger, and a nuclear envelope can be seen. Mature (stage III) oocytes are in late vitellogenesis, yellow in color and filled with yolk globules. The vitelline membrane is obviously divided from the yolk. Ripe (stage IV) 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 (Fig. 4). Female fecundity was determined as clutch size, the combined number of Stage III and IV oocytes present in Stage III or IV ovaries. 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 (Tarver and Tarver, 2014) and confirmed using CellSens software. The diameters of ten oocytes per developmental stage per female were measured, and the mean measurements for each stage were calculated for each female.

Statistical analysis

The total number of female, male, and juvenile (unsexed) fish were calculated for each monthly collection. Chi square analysis was used to test for a significant difference between the number of males and females collected monthly. The equation $W = aL^b$ has been frequently used to express the relationship between length (L) and body weight (W) for numerous species of fishes. A log linear regression model using $\log_{10} W = \log_{10} a + b \cdot \log_{10} L$ (W is the weight of each fish in g, L is each fish's length in mm, log "a" is the y-intercept, and b is the coefficient) was used to determine the relationship between standard length (SL) and body weight (W) for the species in this study. A linear regression analysis displaying $\log_{10}$ W against $\log_{10}$ SL produces a line with slope b and a Y-intercept of $\log_{10}$ a. Mean monthly GSI, total oocytes, and clutch size were evaluated for adult females from May, 2013 to July, 2014, and mean monthly GSI for males. Monthly mean diameter measurements were calculated for Stage I, Stage II, Stage III, and Stage IV oocytes. To determine if statistically significant differences existed between monthly values for GSI and clutch size, one-way ANOVAs tests were performed. 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 using the algorithm of Gleason (1999) (Vasavada, 2016).

Results

Study site temperature and discharge data

Water temperature began to rise in February from a low of 8º C in January to a high of 24.5º C in July. River discharge was high but variable in late winter and early spring before dropping to typical summer low flow (Fig. 1).

Fish Collections

Monthly collections of *Hybopsis amblops* were made from May, 2013, through July, 2014. Few fish were collected in January (no females) and February (one female) due to sustained high river levels. A total of 81 females, 94 males and 78 juveniles were collected. Males were found to reach sexual maturity at a standard length (SL) greater than 49 mm and females at a SL greater than 47 mm. Therefore, specimens with a SL less than 47 mm were classified as a juvenile. Chi square was performed to test for a significant difference between the number of males and females from the expected 1:1 ratio in each collection. A significantly larger number of males were collected in August and November, 2013. No additional significant differences in the sex ratio were revealed within the collection (Table 1).

A relationship between length (L) and body weight (W) has been shown for numerous species of fishes. This relationship was examined in Bigeye Chubs. The length of juveniles (78) varied from 29.43 mm to 47.94 mm with a median of 41.24±4.23 SD mm, and a body mass between 0.27 g to 2.70 g. Sexually mature females (81) ranged from 47.70 mm to 77.20 mm SL with a median of 62.50±5.66 SD mm, and from 1.00 g to 5.90 g in body mass. Males (94) ranged from 49.30 mm to 74.40 mm SL with a median of 61.20±5.81 SD mm, and from 1.50 g to 5.10 g in body mass. A linear regression analysis showed the standard length-weight relationship indicated a good fit, R^2=0.860 (Fig 5).

Reproductive schedule

Average monthly (GSI) was evaluated for males and females from May, 2013 to July, 2014. GSI values began to rise in February (1 female, 2 males), peaked in April (5 females, 6 males) and May (16 females, 24 males) of 2014. GSI values showed a steep decline from May to June (9 females, 15 males), 2014, for both sexes. ANOVA and post-hoc Tukey-Kramer tests showed a significant difference for females ($F_{13,74}$ = 15.54 $p<0.05$) and males ($F_{14,82}$ = 12.11 $p<0.05$) (Fig 6).

Ovarian development, oocyte counts, and oocyte diameters

The developmental stage of each ovary was assessed and recorded in the monthly samples (Table 2). The highest number of oocytes (mean 1,271±375 SD) was found in March, 2014 (7 females) (Table 3A). Stage I oocytes peaked in March at 404 oocytes, but the one specimen collected in February, 2014 contained 1,083 Stage I oocytes. Stage II oocytes peaked in March (mean 630±188 SD) from 7 female specimens. In April number of Stage III and Stage IV oocytes peaked, mean 754±138 SD and mean 58±12 SD respectively. The diameter size of ten oocytes in each stage of maturation were measured from each female. Stage I egg diameters ranged from 0.32 to 0.49 mm, Stage II diameters ranged from 0.38 to 0.67 mm, Stage III ranged from 0.50 to 1.05 mm, and Stage IV varied 0.78 to 1.19 mm. The mean ±SD diameter measurements for each maturation stage was determined per month of collection (Table 3B). The largest oocytes for all four stages were found in April (5 females) and May (16 females). Mean diameter measurements were 0.46±0.05 SD mm for Stage I, 0.61±0.11 SD mm for Stage II, 0.91±0.16 SD mm for Stage III, and 1.05±0.22 SD mm for Stage IV oocytes collected in April. These measurements were not significantly different in the month of May.

Clutch size

Females with Stage III or IV ovaries carrying Stage III or IV oocytes were found in March (7 females), April (5 females) and May (16 females). Mean clutch size was highest in April (5 females) at 812. A one-way ANOVA found a significant difference between the three months ($p<0.01$) and a post-hoc Tukey test found that March (7 females), with the smallest mean clutch size, was significantly different from April (5 females), ($p< 0.01$) but not from May (16 females). Oocyte counts in May were not significantly different from April. The largest individual clutches found were in April (1,102) and in May (1,340). All ovaries examined in June were Stage V, post-spawning, even though they still contained some mature oocytes.

Monthly mean water temperatures (ºC) and oocyte counts were applied to the same graph. The graph revealed a trend: during months with increased water temperatures, oocyte counts were low. Water temperatures were lower in November when oocyte counts began increasing. February to May of 2014, water temps were low but rising as oocyte counts were cresting. In June of 2013 and 2014, the number of oocytes in each female decreased as water temperatures climaxed (Fig 7).

Discussion

The Bigeye Chub in Alabama primarily spawns in April and May as indicated by GSI, ovarian condition and clutch size. This is slightly earlier than the late spring and early summer prediction of Boschung and Mayden (2004). Oocyte size also peaked in April and May. During this spawning peak, water temperature was in the 17 – 21 °C range, and stream discharge was dwindling from the typical early spring high levels found in the Flint River (described more fully in Hodgskins et al., 2016).

A comprehensive model using various strategies was proposed by Winemiller and Rose (1992), later revised by Winemiller (2005), to illustrate common patterns of diversity. This model forms a triangle with the internal angles representing three fundamental strategies labeled opportunistic, equilibrium, and periodic. Each external side of the triangle correlates each strategy to certain ecological conditions. The position of Bigeye Chubs in the trilateral scheme falls between opportunistic (early maturing, low survivorship, small clutches) and periodic (late maturity, low survivorship, large clutches). Females were found with small oocytes in multiple developmental stages and carrying clutches for more than two months, which is good support for viewing the species as a multiple spawner *sensu* Heins and Rabito (1986). As described by Mims and Olden (2013), the species is responding to a flow regime with defined seasonality as well as predictability of flow and resources. This is consistent with our lab group's observations of conditions in the Flint River (Hodgskins et al., 2016).

North American stream leucisids use a range of reproductive strategies and seasons. One uncommon strategy used by some species such as the Texas Shiner (*Notropis amabilis*, (Girard)) is a protracted spawning season, nine months in the case of the Texas Shiner, consistent with the species' utilization of spring systems on the Edwards Plateau with stable water temperatures (Craig et al., 2017). The rheophilic *Notropis photogenis* in the Flint River is among the first to spawn in the river's leucisid community, apparently stimulated to spawn by flood pulses in the late winter and very early spring as water temperature reaches about 12 °C. Females release a large number of small, buoyant oocytes into high water (Hodgskins et al., 2016). What we observed in the Bigeye Chub for spawning season is more typical of stream leucisids, with a peak spawning season in mid-spring. A similar study of the Scarlet Shiner in the Flint River found evidence of a more protracted spawning season from April to July with a May peak, a time period typically with higher temperature and lower discharge (Stallsmith et al., 2018). Another Flint River leucisid, the Whitetail Shiner, *Cyprinella galactura* (Cope), spawns in the summer, June to August (personal observation). The Whitetail Shiner is also different from the other species mentioned since they are crevice spawners with males tending the developing eggs. Our work defining some species reproductive traits of the Bigeye Chub contributes to understanding the community ecology of, in particular, the Flint River of Alabama, which is rich in leucisid species.

The reproductive traits of Bigeye Chubs are similar to Blotched Chubs (*Erimystax insignis)*, a well-studied leucisid in the Flint River. Blotched Chubs have a long spawning period from March to June, peaking in April, as indicated by: GSI, ovarian condition, and presence of clutches in females. Mean clutch sizes found in Blotched Chubs were much smaller than those observed in Bigeye Chubs. The mean number of oocytes found in female Blotched Chubs was 125 during the month of April, and female Bigeye Chubs averaged 800 oocytes in April. Stage IV oocytes were also smaller, averaging 0.74 mm compared to over 1 mm in the Bigeye Chub (Stallsmith et al., 2015). Earlier captive breeding work with Blotched Chubs found that the species sheds demersal eggs over coarse gravel and larvae are benthic, suggesting a need for clean substrate for successful reproduction (Conservation Fisheries, Inc., 2001).

Conclusion

Our data give a good indication of the timing of reproduction by the Bigeye Chub, and fecundity. But we did not attempt to observe their spawning in nature, or keep them in aquaria to observe spawning. Various online searches lead us to conclude that the spawning habits of six recognized *Hybopsis* species are unknown. The Fishtraits Database of

Frimpong and Angermeier (2013) states that the species is likely a lithophilic spawner. The mature oocytes observed are denser than water. *Hybopsis* spawning may be simply releasing demersal eggs into the water column over gravel or cobble, and leaving the eggs to sink to the bottom as found with sympatric Blotched Chubs (Conservation Fisheries, Inc., 2001). This remains to be determined.

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Table 1

Sex distribution of Bigeye Chubs collected monthly from the Flint River from May, 2013 to July, 2014. Chi square was performed to test for a significant difference between the number of males and females in each collection.

No female specimens were located or collected in January, 2014. Only one female specimen was collected in July, 2013, and February, 2014.

*A significantly larger number of males were collected in August and November, 2013.2013 to July, 2014. P-values at α = 0.05.

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Table 2

Monthly summary of female ovarian maturity by stage in female Bigeye Chubs collected from the Flint River in Alabama from May, 2013 to July, 2014.

All ovaries observed in specimens collected from September and October were found to be fully regressed, so no data are reported for those months.

No female specimens were located or collected in January, 2014. Only one female specimen was collected in July, 2013, and February, 2014.

Table 3

A) Monthly mean number of oocytes ±SD per stages I, II, III, and IV found in female Bigeye Chubs collected from the Flint River in Alabama during the time period from May, 2013 to July, 2014.
Latent oocytes were not included in this study. Females collected in August to December of 2013 contained only latent oocytes.
*No female specimens were located or collected in January, 2014. Only one female specimen was collected in July, 2013 (unable to examine oocytes), and February, 2014.

B) Monthly mean±SD diameters (mm) of 10 oocytes from each female ovary at each developmental stage found in Bigeye Chubs collected from the Flint River in Alabama from May, 2013 to July, 2014.
Latent oocytes were not included in this study. Females collected in August to December of 2013 contained only latent oocytes.
*No female specimens were located or collected in January, 2014. Only one female specimen was collected in July, 2013 (unable to examine oocytes), and February, 2014.

Figure 1

A) Monthly water temperatures and average rate of river discharge (ft^3/sec) in the Flint River from May, 2013 to July, 2014. Temperatures were measured at the river during each monthly fish collection, and river discharge data are from an automated station 2 km downstream. Rate of discharge is reported as ft^3/sec because that is how it is reported by the United States Geological Survey (USGS).
B) Graph comparing flow data from this collection period with historical flow data obtained from the USGS databank.

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Figure 2

Map of the Bigeye Chub collection site located north of the Oscar Patterson Road crossing (34˚48’24” N, -86˚28’21” W) on the Flint River in Madison County, Alabama. All fish were collected in a 300 m stretch of the river between May, 2013 and July, 2014.

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Figure 3

The five stages of ovarian maturation from ovaries found in female Bigeye Chubs collected from the Flint River in Alabama from May, 2013 to July, 2014. Stage I - early maturing, Stage II - late maturing, Stage III - mature, Stage IV - ripe, Stage V - post-spawning.

Figure 4

The four stages of oocyte maturation from ovaries found in female Bigeye Chubs collected from the Flint River in Alabama from May, 2013 to July, 2014. From top to bottom: Stage 1 - early maturing, Stage II - late maturing, Stage III - mature, Stage IV - ripe.

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Figure 5

Standard length - weight relationship between 81 female, 94 male, and 78 juvenile Bigeye Chubs collected from the Flint River in Alabama between May, 2013 and July, 2014.

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Figure 6

A) Average monthly gonadosomatic index (GSI) of female Bigeye Chubs collected from the Flint River in Alabama between May, 2013 and July, 2014. No female specimens were collected in January, 2014. Only one female specimen was collected in July, 2013, and February, 2014.
B) Results of one-way ANOVA and Tukey-Kramer test showing significant differences in monthly GSI values across the collection period or female specimens. Months not connected by the same letter are significantly different from one another.
C) Average monthly GSI of male Bigeye Chubs collected from the Flint River in Alabama between May, 2013 and July, 2014.
D) Results of one-way ANOVA and Tukey-Kramer test showing significant differences in monthly GSI values across the collection period for male specimens. Months not connected by the same letter are significantly different from one another.

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Figure 7

Trends for water temperatures (ºC) and oocyte counts. Water temperatures were lower in November when oocyte counts began increasing. February to May of 2014, water temps were low but rising as oocyte counts were cresting. In June of 2013 and 2014, the number of oocytes in each female decreased as wate r temperatures climaxed.
*No female specimens were collected in January, 2014, so no water temperature or oocyte count was included for this month.

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Figure 1(on next page)

River temperature and discharge rate during the study

A) Monthly water temperatures and average rate of river discharge (ft3/sec) in the Flint River from May, 2013 to July, 2014. Temperatures were measured at the river during each monthly fish collection, and river discharge data are from an automated station 2 km downstream. Rate of discharge is reported as ft3/sec because that is how it is reported by the United States Geological Survey (USGS). B) Graph comparing flow data from this collection period with historical flow data obtained from the USGS databank.

A

Month-Yr.	Water Temp (ºC)	River Discharge Monthly Mean in ft^3/s
May-13	21.9	1,177.0
June-13	25.7	417.5
July-13	26.4	934.6
August-13	25.1	818.8
September-13	22.0	262.4
October-13	14.4	159.6
November-13	15.3	249.7
December-13	13.0	1,140.0
January-14	8.0	898.2
February-14	10.2	1,248.0
March-14	15.6	539.0
April-14	17.2	1,094.0
May-14	21.1	542.2
June-14	24.1	765.6
July-14	24.5	219.8

B

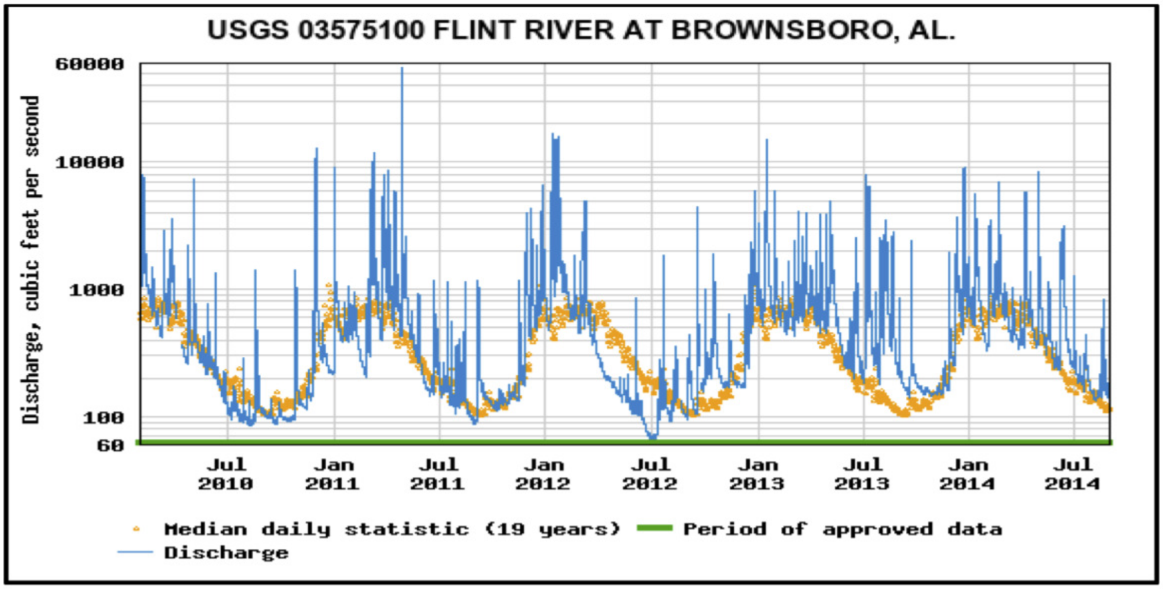

Figure 2(on next page)

Map of the collection site

Map of the Bigeye Chub collection site located north of the Oscar Patterson Road crossing (34˚48’24” N, -86˚28’21” W) on the Flint River in Madison County, Alabama. All fish were collected in a 300 m stretch of the river between May, 2013 and July, 2014.

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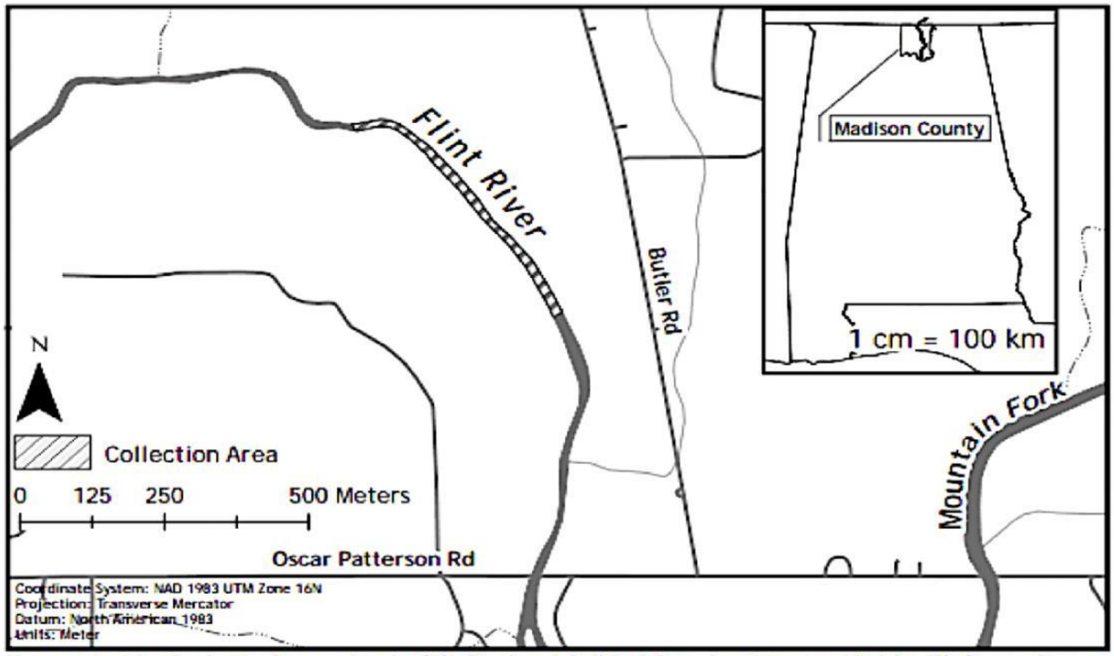

Map created by Benjamin Swan using ArcGIS. Road and Political Bounday data provided by US Census Bureau; Hydrography data provided by USGS

Figure 3(on next page)

Stages of ovarian maturation

The five stages of ovarian maturation from ovaries found in female Bigeye Chubs collected from the Flint River in Alabama from May, 2013 to July, 2014. Stage I - early maturing, Stage II - late maturing, Stage III - mature, Stage IV - ripe, Stage V - post-spawning.

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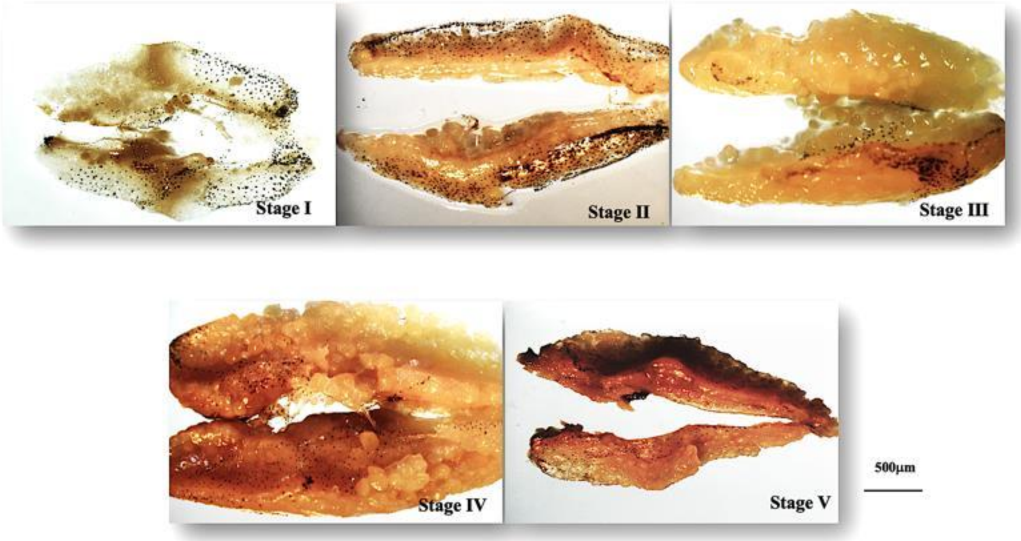
Stage I
Stage II
Stage III
Stage IV
Stage V
500μm

Figure 4 (on next page)

Stages of oocyte maturation

The four stages of oocyte maturation from ovaries found in female Bigeye Chubs collected from the Flint River in Alabama from May, 2013 to July, 2014. From top to bottom: Stage 1 - early maturing, Stage II - late maturing, Stage III - mature, Stage IV - ripe.

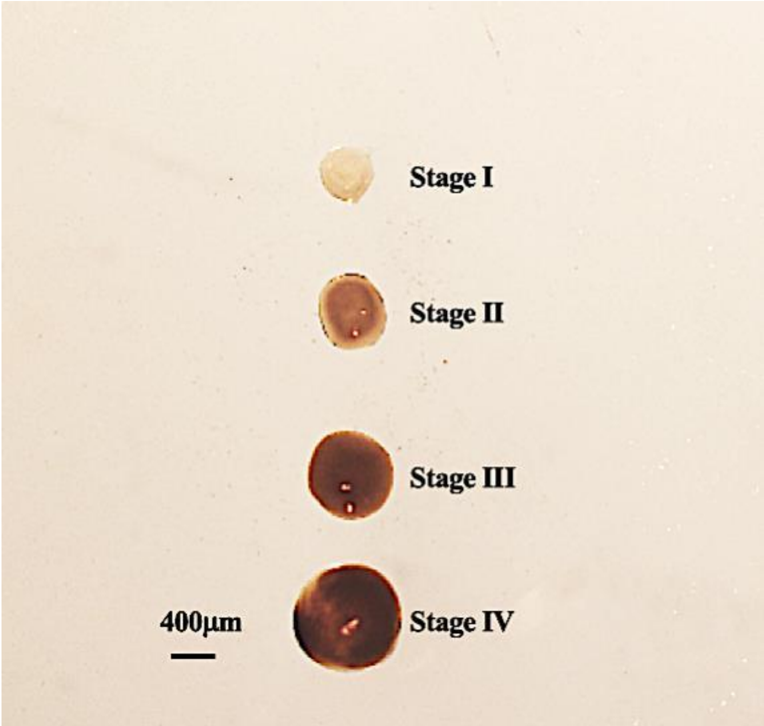
Stage I
Stage II
Stage III
400µm
Stage IV

Figure 5 (on next page)

Length - weight relationship

Standard length - weight relationship between 81 female, 94 male, and 78 juvenile Bigeye Chubs collected from the Flint River in Alabama between May, 2013 and July, 2014.

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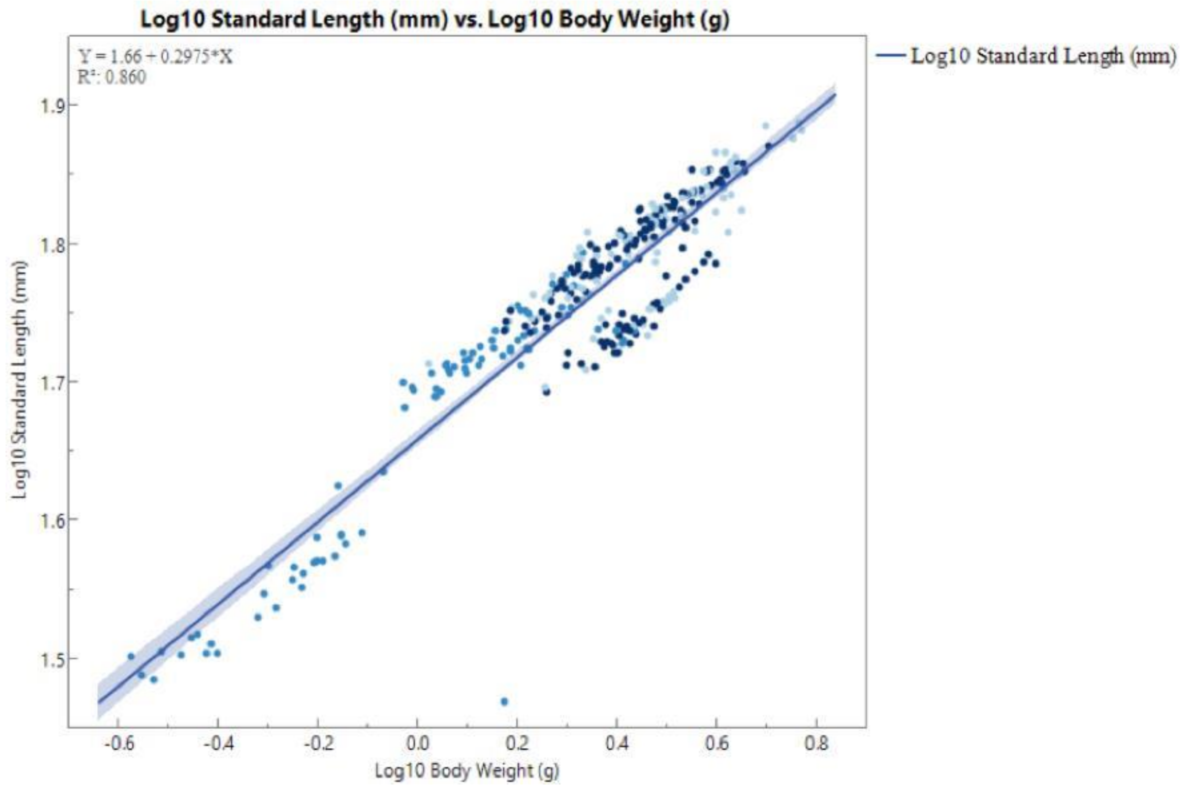
Log10 Standard Length (mm) vs. Log10 Body Weight (g)
Y = 1.66 + 0.2975*X
R²: 0.860
Log10 Standard Length (mm)
1.9
1.8
1.7
1.6
1.5
-0.6
-0.4
-0.2
0.0
0.2
0.4
0.6
0.8
Log10 Body Weight (g)
Log10 Standard Length (mm)

Figure 6 (on next page)

Female GSI data and analysis

A) Average monthly gonadosomatic index (GSI) of female Bigeye Chubs collected from the Flint River in Alabama between May, 2013 and July, 2014. No female specimens were collected in January, 2014. Only one female specimen was collected in July, 2013, and February, 2014. B) Results of one-way ANOVA and Tukey-Kramer test showing significant differences in monthly GSI values across the collection period or female specimens. Months not connected by the same letter are significantly different from one another. C) Average monthly GSI of male Bigeye Chubs collected from the Flint River in Alabama between May, 2013 and July, 2014. Results of one-way ANOVA and Tukey-Kramer test showing significant differences in monthly GSI values across the collection period for male specimens. Months not connected by the same letter are significantly different from one another.

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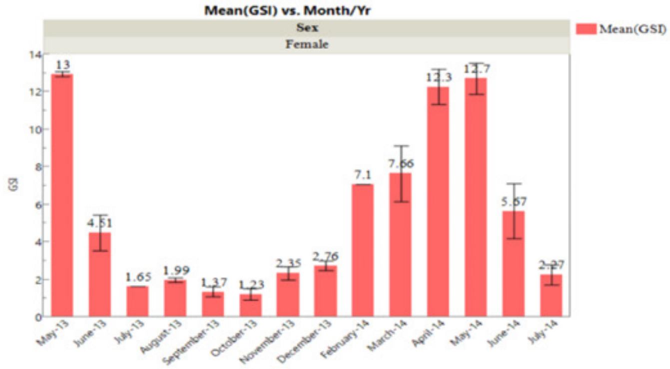
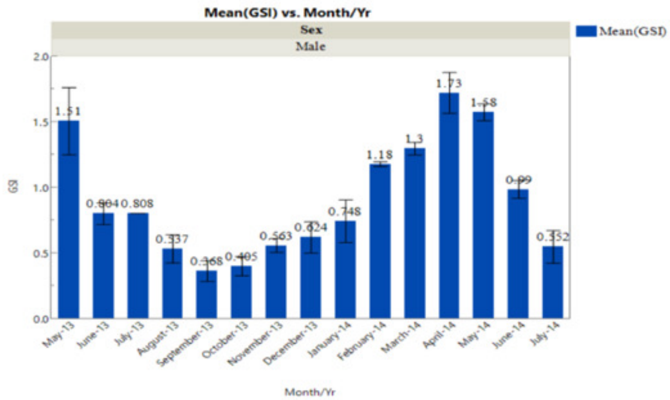
A



B

Level				
May-13	A	B		
May-14	A			
April-14	A	B		
March-14		B	C	
February-14	A	B	C	D
June-14			C	D
June-13			C	D
December-13				D
November-13				D
July-14				D
August-13				D
July-13			C	D
September-13				D
October-13				D

C



D

Level					
April-14	A				
May-14	A	B			
May-13	A	B	C		
March-14	A	B	C	D	E
February-14	A	B	C	D	E
June-14			C	D	
July-13	A	B	C	D	E
June-13				D	E
January-14		B	C	D	E
December-13				D	E
November-13					E
July-14				D	E
August-13					E
October-13				D	E
September-13					

Figure 7 (on next page)

Trends for water temperatures and oocyte counts

Trends for water temperatures (ºC) and oocyte counts. Water temperatures were lower in November when oocyte counts began increasing. February to May of 2014, water temps were low but rising as oocyte counts were cresting. In June of 2013 and 2014, the number of oocytes in each female decreased as wate r temperatures climaxed. *No female specimens were collected in January, 2014, so no water temperature or oocyte count was included for this month.

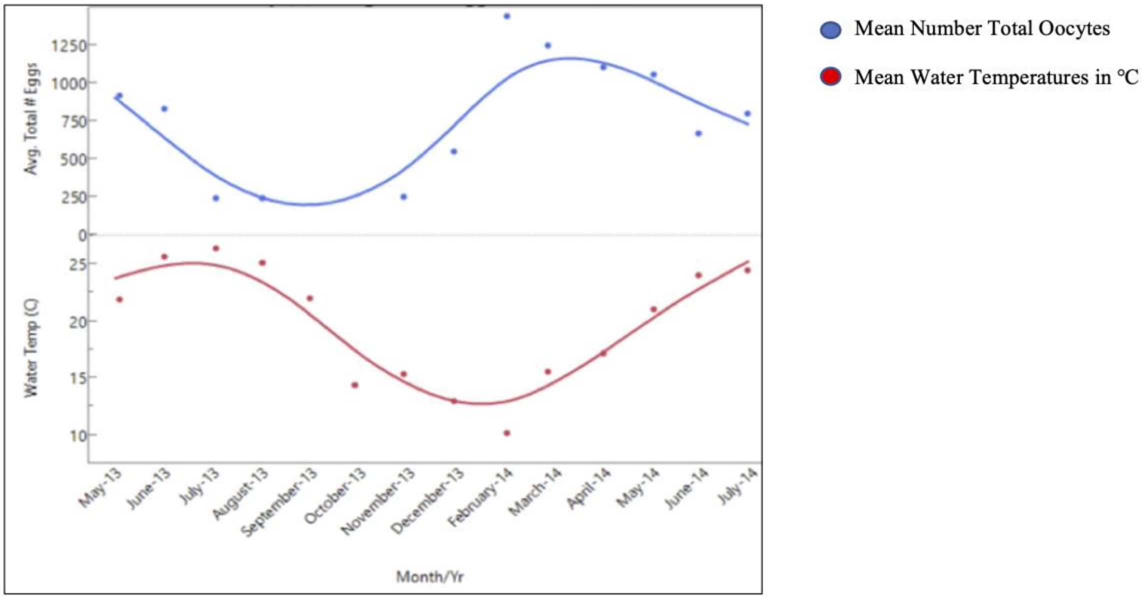

Avg. Total # Eggs
1250
1000
750
500
250
0
Water Temp (C)
25
20
15
10
May-13
June-13
July-13
August-13
September-13
October-13
November-13
December-13
February-14
March-14
April-14
May-14
June-14
July-14
Month/Yr
Mean Number Total Oocytes
Mean Water Temperatures in °C

Table 1 (on next page)

Sex distributions within monthly collections

Sex distribution of Bigeye Chubs collected monthly from the Flint River from May, 2013 to July, 2014. Chi square was performed to test for a significant difference between the number of males and females in each collection. No female specimens were located or collected in January, 2014. Only one female specimen was collected in July, 2013, and February, 2014. *A significantly larger number of males were collected in August and November, 2013.2013 to July, 2014. P-values at $\alpha = 0.05$.

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Month/Yr	Females	Males	Juveniles/Unsexed	Total	p-Value
May-13	2	6	1	9	0.14
June-13	10	20	5	35	0.06
July-13	1	1	0	2	0.00
August-13	7	17	26	50	0.04*
September-13	5	10	1	16	0.19
October-13	4	5	2	11	0.73
November-13	6	15	6	27	0.04*
December-13	11	5	10	26	0.13
January-14	0	2	0	2	0.00
February-14	1	2	13	16	0.56
March-14	7	2	5	14	0.09
April-14	5	6	9	20	0.76
May-14	16	24	3	43	0.20
June-14	9	15	0	24	0.22
July-14	10	7	3	20	0.46

Table 1: Sex distribution of Bigeye Chubs collected monthly from the Flint River from May, 2013 to July, 2014. Chi square was performed to test for a significant difference between the number of males and females in each collection.
No female specimens were located or collected in January, 2014. Only one female specimen was collected in July, 2013, and February, 2014.
*A significantly larger number of males were collected in August and November, 2013.2013 to July, 2014. P-values at $\alpha = 0.05$.

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Table 2 (on next page)

Monthly summary of female ovarian maturity

Monthly summary of female ovarian maturity by stage in female Bigeye Chubs collected from the Flint River in Alabama from May, 2013 to July, 2014. All ovaries observed in specimens collected from September and October were found to be fully regressed, so no data are reported for those months. No female specimens were located or collected in January, 2014. Only one female specimen was collected in July, 2013, and February, 2014.

Month/Yr	# of Stage I Ovaries	# of Stage II Ovaries	# of Stage III Ovaries	# of Stage IV Ovaries	# of Stage V Ovaries
May-13	0	0	0	2	0
June-13	0	0	0	2	4
July-13	0	0	0	0	1
August-13	0	0	0	0	3
September-13	0	0	0	0	0
October-13	0	0	0	0	0
November-13	3	0	0	0	0
December-13	10	0	0	0	0
February-14	0	1	0	0	0
March-14	0	0	5	2	0
April-14	0	0	1	4	0
May-14	0	0	1	10	3
June-14	0	0	0	1	6
July-14	0	0	0	0	3

Table 2: Monthly summary of female ovarian maturity by stage in female Bigeye Chubs collected from the Flint River in Alabama from May, 2013 to July, 2014.
All ovaries observed in specimens collected from September and October were found to be fully regressed, so no data are reported for those months.
No female specimens were located or collected in January, 2014. Only one female specimen was collected in July, 2013, and February, 2014.

Table 3(on next page)

Monthly means of oocyte number and oocyte size.

A) Monthly mean number of oocytes ±SD per stages I, II, III, and IV found in female Bigeye Chubs collected from the Flint River in Alabama during the time period from May, 2013 to July, 2014. Latent oocytes were not included in this study. Females collected in August to December of 2013 contained only latent oocytes. *No female specimens were located or collected in January, 2014. Only one female specimen was collected in July, 2013 (unable to examine oocytes), and February, 2014. B) Monthly mean±SD diameters (mm) of 10 oocytes from each female ovary at each developmental stage found in Bigeye Chubs collected from the Flint River in Alabama from May, 2013 to July, 2014. Latent oocytes were not included in this study. Females collected in August to December of 2013 contained only latent oocytes. *No female specimens were located or collected in January, 2014. Only one female specimen was collected in July, 2013 (unable to examine oocytes), and February, 2014.

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A

Month/Yr	Mean# Stage I Oocytes	Mean# Stage II Oocytes	Mean# Stage III Oocytes	Mean# Stage IV Oocytes	Total Mean# Oocytes
May-13	44±10	388±130	422±152	8±1	861±193
June-13	184±46	392±142	257±59	30±4	832±118
February-14	1083*	164*	0*	0*	1247±231
March-14	404±132	630±188	219±47	32±2	1271±375
April-14	253±64	164±42	754±138	58±12	1230±128
May-14	240±60	359±133	425±193	49±3	1073±191
June-14	236±60	275±61	131±33	41±9	669±129
July-14	72±22	129±25	574±172	39±3	796±142

B

Month/Yr	Mean Diameter of Stage I Oocytes (mm)	Mean Diameter of Stage II Oocytes (mm)	Mean Diameter of Stage III Oocytes (mm)	Mean Diameter of Stage IV Oocytes (mm)
May-13	0.44±0.05	0.52±0.11	0.79±0.16	1.01±0.22
June-13	0.43±0.05	0.55±0.11	0.73±0.16	0.91±0.22
February-14	0.44*	0.49*	0.00*	0.00*
March-14	0.45±0.05	0.58±0.11	0.76±0.16	0.99±0.22
April-14	0.46±0.05	0.61±0.11	0.91±0.16	1.05±0.22
May-14	0.47±0.05	0.61±0.11	0.92±0.16	1.03±0.22
June-14	0.43±0.05	0.57±0.11	0.74±0.16	0.94±0.22
July-14	0.39±0.05	0.45±0.11	0.70±0.16	0.92±0.22

Table 3:

A) Monthly mean number of oocytes ±SD per stages I, II, III, and IV found in female Bigeye Chubs collected from the Flint River in Alabama during the time period from May, 2013 to July, 2014.
   Latent oocytes were not included in this study. Females collected in August to December of 2013 contained only latent oocytes.
   *No female specimens were located or collected in January, 2014. Only one female specimen was collected in July, 2013 (unable to examine oocytes), and February, 2014.

B) Monthly mean±SD diameters (mm) of 10 oocytes from each female ovary at each developmental stage found in Bigeye Chubs collected from the Flint River in Alabama from May, 2013 to July, 2014.
   Latent oocytes were not included in this study. Females collected in August to December of 2013 contained only latent oocytes.
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