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Reproductive biology of *Hybopsis amblops*, the Bigeye Chub, in the Flint River of Alabama

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Abstract

The purpose of this study was to establish a reproductive schedule and examine reproductive traits that shape fecundity of the Bigeye Chub, *Hybopsis amblops* Leuciscidae in the Flint River system of north Alabama. Traits associated with reproduction, growth, and maturation were assessed. Specimens were collected and examined monthly from August, 2013 until July, 2014. This Bigeye Chub population primarily spawns in April and May as indicated by gonadosomatic index (GSI), ovarian condition and clutch size. Mean GSI values began to rise in February, peaking in April and May at over 13% for females and 1.6% for males. There was a steep decline in these values from May to June for both sexes. Clutch sizes ranged from 550 to 2,566 oocytes, with females of mean 62.5 mm standard length. The largest mean clutch size was found in specimens collected in March, 2014 at 1,271. Mature oocytes, Stage IV, were relatively small in comparison to other leucisids found in the Flint River with a mean diameter of approximately 1 mm. The collection of this information is 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.

Keywords

ovarian maturation, oocyte maturation, Leuciscidae, river fishes

Cover Page Footnote

We would like to thank Kelly Hodgskins, Kara Million, and Josh Mann for help with field work.

INTRODUCTION

Hybopsis amblops (Rafinesque, 1820), 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, 2025). They are currently thought to be extirpated in the Missouri River drainage, and rarely found in segments of the Illinois River drainage in Illinois. 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 (NatureServe, 2012). 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. 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).

Bigeye Chubs 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 Bigeye Chub populations and insufficient data concerning their reproductive biology inspired this study. While data shown here are limited to a 12-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 Bigeye Chub in the Flint River system of north Alabama, which is at the southern edge of its range. Life history traits associated with reproduction, growth, and maturation were assessed. We also wished to compare Bigeye Chubs to sympatric leucisids in the Flint River that have been similarly studied including *Erimystax insignis* (Hubbs and Crowe, 1956), Blotched Chub (Stallsmith et al., 2015); *Notropis photogenis* (Cope, 1867), Silver Shiner (Hodgskins et al., 2016); and *Lythrurus fasciolaris* (Gilbert, 1891), 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. 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 (Figure 1). Collections were made monthly from August 2013–July 2014. Female specimens were not available for examination in January, 2014. Only one female specimen was available for examination in 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 with a radius of 2.5-m and a 6-mm mesh was used occasionally. All fish were euthanized on site using 10 ml of 1:10 clove oil: 95% ethanol solution diluted with 350 ml river water. Samples were then placed in 10% phosphate buffered formalin for tissue fixation and storage. Water temperature was taken during each collection with an alcohol thermometer.

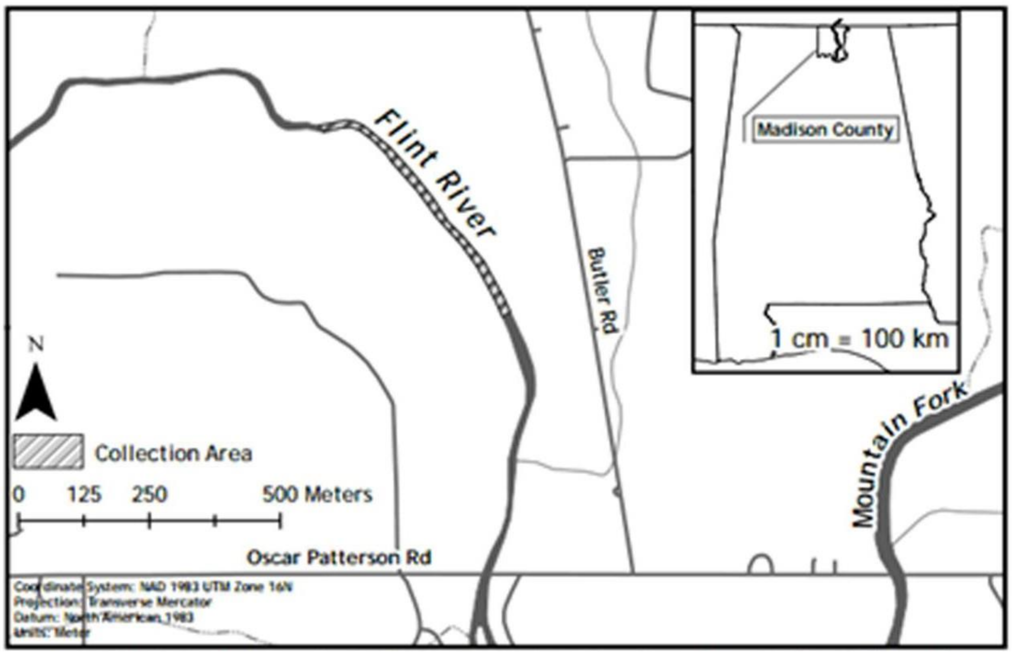

Figure 1. Map of the Flint River collecting site off of Oscar Patterson Road, Madison County, Alabama. Map created by Benjamin Swan using ArcGIS. Road and political boundary data provided by US Census Bureau. Hydrography data provided by USGS.

Laboratory analysis

Standard length (SL) of each individual was measured with a digital caliper to the nearest 0.01 mm. Gross body mass was obtained to the nearest 0.01-g with an OHAUS Explorer (Parsippany, New Jersey) digital balance after excess fluid was blotted from the individual's body. The sex of each fish was determined by excision and examination of gonadal tissue under an Olympus SZX7 (Breinigsville, Pennsylvania) dissecting microscope. After excess surface fluid was blotted 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 (Breinigsville, Pennsylvania) camera. The images were later analyzed for maturation status and number using the cellSens Standard software (Ver. 1.5) provided by Olympus with camera purchase. Each image was captured at 6.4x 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 were small in size, usually opaque, and contain only latent oocytes. Maturing (Stage II) ovaries were larger, inhabiting a larger portion of the abdominal cavity, and contain various sizes of white to cream colored oocytes. Advanced maturation (Stage III) ovaries were bulkier and densely packed with oocytes. The oocytes were yellow to orange, and various sizes of vitellogenic oocytes were visible in between oocytes that were ready to be released during spawning. Ripe (Stage IV) ovaries were partially ovulated and oocytes were released when squeezing the sides of the fish. In Stage IV the ovary has obtained maximal development, but vitellogenic oocytes of several sizes were present in between the mature oocytes making possible multiple spawning. Spawned and recovering (Stage V) ovaries were still relatively large and flaccid with remaining empty spaces, but contained different sizes of developing vitellogenic oocytes (Figure 2). This stage can occur in between spawning cycles, and is indicative of the end of the spawning season.

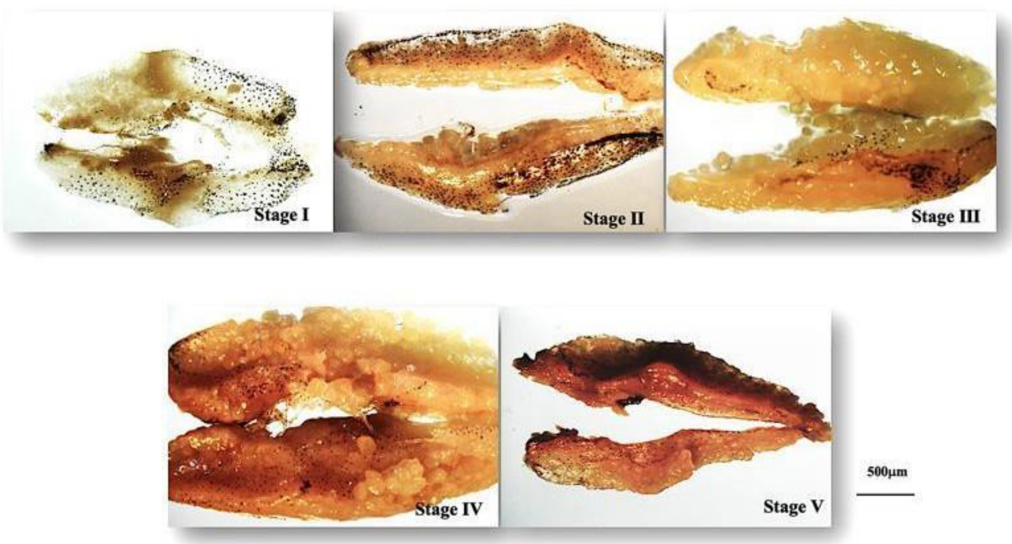

Figure 2. The five stages of ovarian maturation from ovaries found in female Bigeye Chubs collected August 2013 – July 2014 from the Flint River, Alabama. Stage I – early maturing, Stage II – late-maturing, Stage III – mature, Stage IV – ripe, and Stage V – post-spawning.

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 (Figure 3). Female fecundity was determined as clutch size, and was 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.

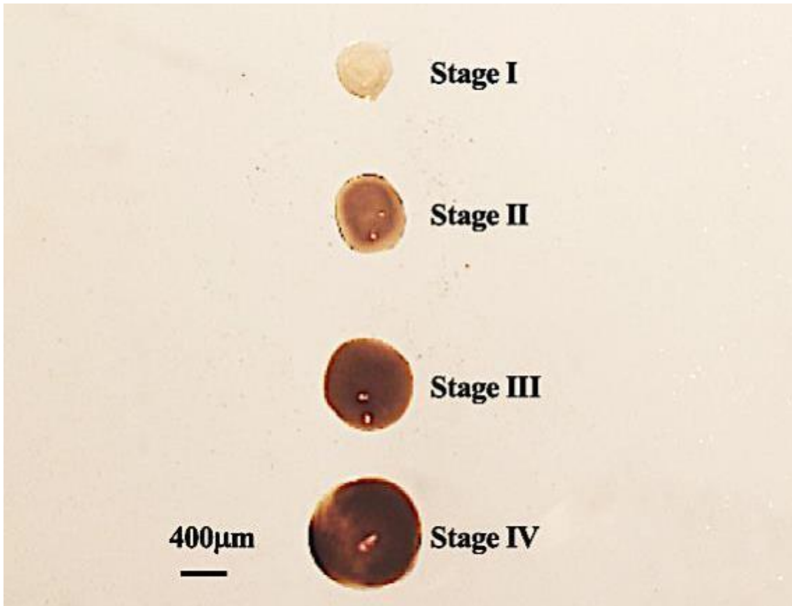

Figure 3. The four stages of oocyte maturation from ovaries found in female Bigeye Chubs c collected August 2013 – July 2014 from the Flint River, Alabama. From top to bottom: Stage I – early maturing, Stage II – late maturing, Stage III – mature, and Stage IV – ripe.

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 customized program in Microsoft Visual Studio, and confirmed cellSens Standard software (Ver. 1.5) as in Stallsmith et al. (2018). 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. Mean monthly GSI, total oocytes, and clutch size were evaluated for adult females from August 2013–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 ANOVA tests were performed. Tukey-Kramer Honestly Significant Difference 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).

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 (Table 1).

Table 1. Monthly water temperatures and average rate of river discharge August 2013 – July 2014 from the Flint River, Alabama.

Month	Water Temperature (°C)	Average Monthly River Discharge (ft^3/sec)
August	25.1	819
September	22	262
October	14.4	160
November	15.3	250
December	13	1140
January	8	899
February	10.2	1248
March	15.6	539
April	17.2	1094
May	21.1	542
June	24.1	766
July	24.5	220

Fish Collections

Few Bigeye Chubs were collected in January (no females) and February (one female) due to sustained high river levels. Bigeye Chubs collected included 81 females, 94 males and 78 juveniles. Bigeye Chub males were found to reach sexual maturity at >49-mm SL and females at >47-mm SL. Therefore, specimens <47-mm SL were classified as a juvenile. The length of juvenile Bigeye Chubs varied from 29.4–47.94-mm with a median of 41.24 ± 4.23 SD mm and a body mass between 0.27 to 2.70 g. Sexually mature female Bigeye Chubs ranged from 47.70 to 77.20 mm SL with a median of 62.50 ± 5.66 SD mm and from 1.00 to 5.90 g in body mass. Male Blotched Chubs ranged from 49.30 to 74.40 mm SL with a median of 61.20 ± 5.81 SD mm and from 1.50 to 5.10 g in body mass.

Reproductive schedule

Average monthly GSI was evaluated for Bigeye Chub males and females from August 2013–July 2014. GSI values began to rise in February (1 female, 2 males) and peaked in April (5 females, 6 males) and May (16 females, 24 males). GSI values showed a steep decline from May to June (9 females, 15 males) for both sexes (Figures 4A, 4B).

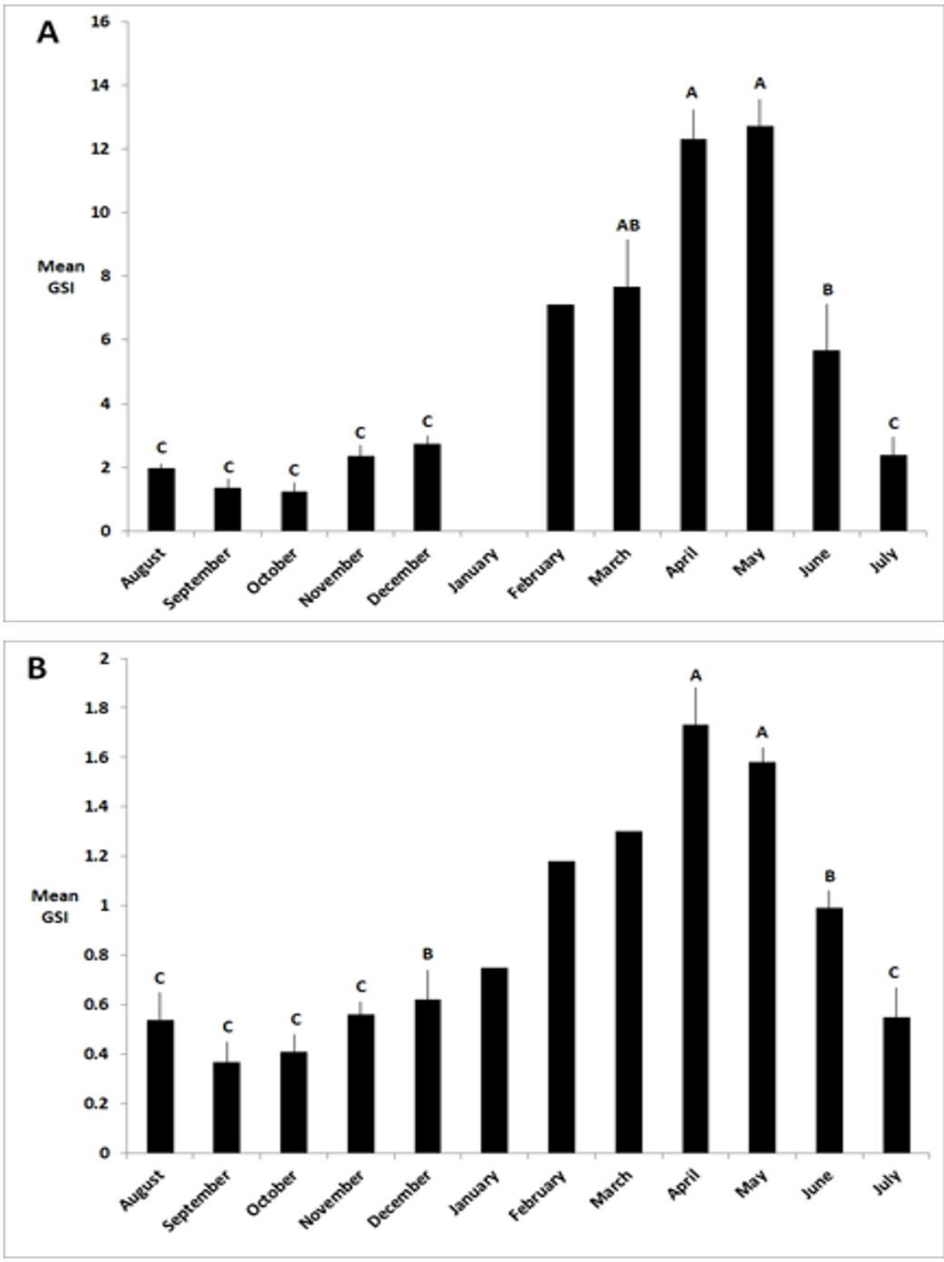

Figure 4. Average monthly gonadosomatic index (GSI) of (A) female and (B) male Bigeye Chubs collected August 2013 – July 2014 from the Flint River, Alabama. No female specimens were collected in January, 2014. Only one female specimen was collected in February, 2014. Lines above bars represent 1 SE. Results based on one-way ANOVA and Tukey-Kramer post hoc test. Months with different letters are significantly different from one another.

Ovarian development, oocyte counts, and oocyte diameters

The developmental stage of each Bigeye Chub ovary was assessed and recorded in the monthly samples. Ovarian regression had become so advanced in September and October that it was impossible to characterize ovarian development. March, April, and May had the most mature ovaries (Table 2). The highest number of oocytes (mean 1,271 ± 375 SD) was found in March (7 females) (Table 3). The mean number of Stage I oocytes peaked in March at 404 oocytes, but the single specimen collected in February contained 1,083 Stage I oocytes. Stage II oocytes peaked in March (mean 630 ± 188 SD) from 7 female specimens. In April, the number of Stage III and Stage IV oocytes peaked with a mean of 754 ± 138 SD and 58 ± 12 SD respectively.

Table 2. Monthly numbers of mature ovaries by stage from Bigeye Chubs collected August 2013 – July 2014 from the Flint River, Alabama.

Month	Stage I	Stage II	Stage III	Stage IV	Stage V
August					2
November	3				
December	10				
February		1			
March			5	2	
April			1	4	
May			1	12	4
June					6
July					3

Table 3. Mean number of oocytes per developmental stage (Stages I – IV) and total number of oocytes found per month for female Bigeye Chubs collected August 2013 – July 2014 from the Flint River, Alabama. The number of females examined per month is indicated within parentheses.

Month (sample size)	Stage I	Stage II	Stage III	Stage IV	Total
February (n = 1)	1083	164	0	0	1247
March (n = 7)	404	630	219	32	1285
April (n = 5)	253	164	754	58	1229
May (n = 16)	240	359	425	49	1073
June (n = 9)	236	275	131	41	683
July (n = 10)	72	129	574	39	814

The diameter of ten oocytes in each stage of maturation were measured from each Bigeye Chub 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 from 0.78 to 1.19 mm. The mean diameter measurements for each maturation stage was determined per month of collection (Table 4). The largest oocytes for all four stages were found in April (5 females) and May (16 females). Mean diameter measurements were 0.46 mm for Stage I, 0.61 mm for Stage II, 0.91 mm for Stage III, and 1.05 mm for Stage IV oocytes collected in April. These measurements were not significantly different in the month of May.

Table 4. Mean diameter (mm) of female Bigeye Chub oocytes in each developmental stage (stages I-IV). Samples collected August 2013 – July 2014 from the Flint River, Alabama.

Month (sample size)	Stage I	Stage II	Stage III	Stage IV
February (n = 1)	0.44	0.45	n.a.	n.a.
March (n = 7)	0.45	0.58	0.77	1.00
April (n = 5)	0.47	0.62	0.91	1.05
May (n = 16)	0.47	0.62	0.93	1.04
June (n = 9)	0.44	0.58	0.74	0.95
July (n = 10)	0.40	0.46	0.71	0.92

Clutch size

Bigeye Chub 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), and the mean clutch size was highest in April (5 females) at 812. A one-way ANOVA found significant ($p < 0.01$) differences between the three months and a post-hoc Tukey-Kramer test found that March (7 females), with the smallest mean clutch size, was significantly different from April (5 females), 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, although with some mature oocytes.

DISCUSSION

Bigeye Chubs in Alabama primarily spawn in April and May as indicated by GSI, ovarian condition, oocyte size, and clutch size. This timing is slightly earlier than the prediction of late spring and early summer of Boschung and Mayden (2004). During this spawning peak, water temperature was in the 17–21 °C range as stream discharge was dwindling from the typical early spring high levels found in the Flint River (described more fully in Hodgskins et al., 2016). The only comparable study on the Bigeye Chub was conducted in Salt Fork of the Vermilion River (Wabash River drainage) in Illinois found spawning occurred over sandy gravel substrates in shallow water with water temperature in the 18–22 °C range (Tiemann et al., 2022).

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). Female Bigeye Chubs were found with small oocytes in multiple developmental stages and carrying clutches for more than two months, which is support for viewing the species as a multiple spawner *sensu* Heins and Rabito (1986). As described by Mims and Olden (2013), Bigeye Chubs are likely responding to a flow regime with defined seasonality. This observation is consistent with earlier ones of conditions in the Flint River (Hodgskins et al., 2016).

The reproductive traits of Bigeye Chubs are similar to Blotched Chubs, 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 (Stallsmith et al., 2015). Mean clutch sizes found in Blotched Chubs were much smaller than those observed in Bigeye Chubs. Bigeye Chub 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, 1868), spawns in the summer, from June to August (Stallsmith, unpublished data). 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.

Conclusion

Our study provides an indication of the fecundity and timing of reproduction by the Bigeye Chub in comparison to other species. However, we did not attempt to observe their spawning in nature, or keep them in aquaria to observe spawning. 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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