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A Comparison of Seasonal Reproductive Pattern in Two Sympatric Darters of the *Simoperca* Clade, *Etheostoma duryi* and *Etheostoma simoterum*

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Abstract

We present results from an examination of the seasonal reproductive patterns of two closely related sympatric darter species of the *Simoperca* clade, *Etheostoma duryi* and *Etheostoma simoterum.* Most members of the genus *Etheostoma* exhibit striking sexual dimorphism, making reproductive strategy a logical point of study. Monthly collections of specimens over a one-year period were performed at a single site on the Flint River near Huntsville, Alabama. Standard length and gross somatic mass were measured for all individuals. Sex ratio was examined for possible skew. Measures of reproductive effort were monthly means of gonadosomatic index of both sexes, total oocyte count, oocyte size at different development stages, and clutch size. Reproductive season for both species was February through May. *Etheostoma duryi* was found to be slightly larger in SL and mass, and to produce more oocytes, larger clutch size, and smaller oocytes earlier in the breeding season. Both species displayed strong female skew, especially *Etheostoma simoterum.*

Keywords

sexual dimorphism, female skew, gonadosomatic index, clutch size, percidae

Cover Page Footnote

Thanks to Josh Mann and Crissy Tarver for assistance with field work.

INTRODUCTION

Our research examines reproductive pattern in two similar snubnose darter species, *Etheostoma duryi* and *E. simoterum*, that share habitat in many streams of the middle Tennessee Valley of north Alabama. The genus *Etheostoma* in the family Percidae is the largest freshwater fish genus in North America with over 150 species (Near et al., 2011). *Etheostoma duryi* and *E. simoterum* of the clade *Simoperca* (Near et al., 2011, 2016) have similar body structures, sizes, coloration patterns, and diets. The species are often sympatric in low order streams and have very similar life cycles. Both spawn in spring, mature at one year of age and usually live for less than two years, typical of other *Simoperca* species (Johnston and Haag, 1996). Spawning occurs in bedrock pools and crevices, where males and females essentially vibrate together above the crevices in the rocky bottom of the river to release eggs and sperm into the water. The male and female both leave once breeding has occurred, and there is no parental care after the eggs and sperm are deposited (Page and Mayden, 1981). This is similar to the behavior of another *Simoperca* species, *E. raneyi*, from aquarium observations (Johnston and Haag, 1996).

The reproduction of these two species has not been well studied. Reproductive studies have been done on *E. simoterum* with very small sample sizes by Page and Mayden (1981) and Heins (2001). Stallsmith and Bedingfield (2015) conducted a study comparing reproductive pattern of two populations of *E. duryi* and found the spawning season to begin in early March with a peak possibly occurring in April and into May. Our study adds to the previous research by using larger sample sizes and studying the species from a site where they exist in sympatry. To the human eye, the two species are very similar in size and coloration. However, *E. simoterum* is more of a riffle specialist (Boschung and Mayden, 2004) and would be expected to be adapted to a more variable environment. The collection site is unusual in having roughly equal numbers of both species.

We are particularly interested in whether *E. duryi* and *E. simoterum* exhibit differences in measures of reproductive pattern including gonadosomatic index (GSI), clutch size, total number of developing oocytes, and oocyte diameter. We collected standard length (SL) and gross somatic mass data to assess whether one species may be larger than the other. Monthly sex ratios were examined for evidence of skew. We address the hypothesis that the two species have some difference(s) in reproductive strategy.

METHODS

Study site and sampling

Specimens of both species were collected at the Oscar Patterson road crossing (34.8805463, -86.4805820) on the Flint River in the Tennessee River watershed located in Madison County, Alabama. Both *E. simoterum* and *E. duryi* are common at this site. The river is not dammed, and runs through mixed farmland and woodlots. Water depth and flow at this site make it easy to maneuver at almost all times of the year. The river at this site is a third order stream composed of a series of glides and riffles with a substrate primarily of bedrock, cobble, and gravel. The water was less than a half meter deep on sampling dates. Collections were performed at least once per month from September, 2014 through August, 2015. The goal was to collect 30 adults of each species, and if enough fish were not collected in the first trip in a given

month, a second trip was made. Methods for the collection and handling of fish were approved by the University of Alabama in Huntsville Institutional Animal Care and Use Committee.

Fishes were collected by kick-seining. Once the collection process was complete, several ml of a 1:9 clove oil and ethanol mixture were added to about 300 ml of river water in the collecting bucket to humanely euthanize the fishes. Following euthanasia, fishes were placed in phosphate buffered 10% formalin for fixation and storage.

Fish data collection

Species identification was based on coloration, in particular the presence or absence of a window in the last lateral blotch along the side. *Etheostoma simoterum* possesses this window, whereas *E. duryi* does not. Standard length (SL) was measured to the nearest millimeter (mm) using digital calipers. Gross mass was measured to the nearest 0.01 gram (g) on an Ohaus Explorer balance after the specimen was blot dried. Each specimen was placed in a separate small plastic bag. All bags from a single month were placed together in one or more 4L glass jars and stored in 10% phosphate buffered formalin.

Specimens were dissected to determine sex. Individuals of both species smaller than 32 mm SL were found to lack mature gonads, and neither dissected nor included in analyses. Gonadal tissue was excised with the aid of a dissecting microscope. Dissection was performed by cutting from the mouth to the tail along the bottom side of the fish, pinning back the skin and muscular tissue, moving other organs and tissue out of the way in order to identify the gonadal tissue, and removing the tissue using a pin and tweezers. Gonadal tissue was stored in 10% phosphate buffered formalin in 1.5 ml Eppendorf tubes.

Testes and ovaries were imaged using an Olympus SZX7 dissecting microscope equipped with a 12 megapixel digital camera controlled with the CellSens software package, and then weighed. No further examination of testes was performed. Before being imaged or weighed, any connective tissue was removed from the testes or ovaries. Gonadal mass (g) was used along with gross somatic mass to calculate the gonadosomatic index (GSI) for each specimen as follows:

$$GSI = [\text{gonad mass}/(\text{gross somatic mass}-\text{gonad mass})] \times 100$$

Fifteen females if available were examined for each species each month, chosen at random. All oocytes were counted in excised ovaries from digital images. Oocytes were arranged into a single layer the size of the image field on a glass slide, with multiple images made. As many as ten images were needed to capture all of the oocytes from each examined female. Digital photos were taken at a total magnification of 2.2x. For months when mature or ripe ovaries were observed, oocyte maturation stage was determined from captured images following a modification of the scheme used by Heins and Rabito (1986). The original scheme divided oocytes into five stages: latent, early maturing, late maturing, mature, and ripe. We used the following four stages excluding the very small, latent oocytes. Early maturing (stage 1) oocytes are relatively small, less than half the diameter of a mature oocyte, usually white with the nucleus obscured by yolk deposition. Late maturing (stage 2) oocytes are greater than half the diameter of a mature oocyte, more opaque and usually cream in color. Mature (stage 3) oocytes are larger than maturing oocytes, cream or yellow in color, with visible oil droplets and the

vitelline membranes visibly separated from the yolk. Ripe (stage 4) oocytes are visibly larger than mature oocytes, yellow or dark yellow brown in color with the vitelline membranes well separated from a smaller yolk mass. All oocytes that had reached at least the early maturing stage were counted and grouped according to stage using a tool available on the CellSens software package. The program EggHelper (Tarver and Tarver, 2014) was used to count and stage oocytes. Clutch sizes were determined by counting stage 3 and 4 oocytes on the images. Stages 3 and 4 are the stages that have undergone vitellogenesis and are either close to being prepared or fully prepared for spawning and as such their combined number is an indicator of near-term spawning competence and are reported as clutch size (Heins and Rabito, 1986; Million et al., 2017).

Statistical analyses

A repeated measures two way ANOVA was used to test for differences between the two species in monthly mean oocytes count, monthly mean clutch count, and size (diameter) of oocytes in each of the five developmental stages. A repeated measures design is used when multiple observations on the same replicate are collected at different times, as is true of this study's data (Gotelli and Ellison, 2013). In particular, a mixed repeated design was used, with the two species as Factor A (fixed) and the specific measures of oocytes count, clutch count, or size of oocytes as Factor B (repeated). Tests were done using Daniel's XL Toolbox, version 7.3.4.

Analysis of Covariance (ANCOVA) was used to test for monthly relationships between average GSI and clutch size values within each species for each reproductive month. GSI is used as the covariate and clutch size is the response (dependent) variable. The hypothesis in ANCOVA is that the covariate contributes to variation in the response variable. Specifically, the hypothesis being tested is that relatively larger ovaries represent mature reproductive condition in a fish that should influence clutch size. ANCOVA tests for heterogeneity of the slopes of each monthly data set. If no heterogeneity is found, a common slope can be fitted to all treatments and adjusted dependent variable means can be calculated. These adjusted means are what would be expected if all groups had the same covariate score (Gotelli and Ellison, 2013). This test allows an assessment of how clutch size may vary between *E. duryi* and *E. simoterum.* The months of February through May were examined with ANCOVA, since all females examined in each of these months had a clutch size of ten or greater. The specific test performed was a One-Way ANCOVA using an Excel spreadsheet developed and made available by VassarStats (2020). The alpha level used for all statistical analyses was 0.05. All statistical analyses were conducted in Microsoft Excel 2019.

RESULTS

Over a one-year period divided into monthly collections, 112 male *E. duryi*, 158 female *E. duryi*, 203 male *E. simoterum*, and 445 female *E. simoterum* were collected. *Etheostoma simoterum* was consistently more abundant throughout every month of collection. This is thought to be a product of the collection site. *Etheostoma duryi* is typically found more downstream in general distribution than *E. simoterum*. If collections had been conducted a significant distance upstream or downstream from the Oscar Patterson crossing on the Flint River, the ratio of *E. simoterum* to *E. duryi* would likely have been different.

Standard lengths and masses of males and females for each month are shown in Table 1. In monthly averages of both SL and mass, *E. duryi* is consistently but not significantly larger than *E. simoterum*, especially males. However, during dissections it was noticed that *E. simoterum* specimens had more fatty tissue than those of *E. duryi*, especially during months of peak reproduction. More females were caught than males of both species during the February to May breeding season. This season is defined by mean female GSI > 4, and all females carrying a clutch. More females were caught than males of *E. simoterum* throughout the year (Table 2). In the breeding season, the female to male ratio was 2.3:1 for *E. duryi* and 3.4:1 for *E. simoterum.*

Table 1. Mean SL and mass of male and female *Etheostoma duryi* and *E. simoterum* over a 12-month period from September 2014 to August 2015. Standard Error (SE) for each mean appears in parentheses. Because only three *E. simoterum* males were collected in December, 2014, SE is reported as n/a.

	E. duryi Mean SL (mm)		*E. simoterum* Mean SL (mm)		*E. duryi* Mean mass (g)		*E. simoterum* Mean mass (g)	
Month	Male	Female	Male	Female	Male	Female	Male	Female
Sept. 2014	44.71 (1.91)	41.30 (1.69)	39.85 (0.82)	38.91 (1.00)	1.25 (0.17)	1.01 (0.14)	0.93 (0.07)	0.88 (0.08)
Oct. 2014	43.77 (2.83)	39.94 (2.04)	40.41 (0.94)	38.06 (0.84)	1.46 (0.35)	1.02 (0.18)	0.98 (0.09)	0.81 (0.07)
Nov. 2014	43.46 (1.34)	37.16 (1.78)	39.21 (0.87)	38.20 (0.50)	1.16 (0.10)	0.88 (0.14)	0.82 (0.08)	0.78 (0.04)
Dec. 2014	40.43 (2.15)	35.66 (0.83)	38.47 (n/a)	37.23 (0.98)	0.94 (0.14)	0.64 (0.04)	0.81 (n/a)	0.74 (0.07)
Jan. 2015	41.25 (1.02)	38.96 (1.16)	39.74 (0.48)	37.38 (0.76)	1.08 (0.11)	0.88 (0.10)	0.95 (0.04)	0.76 (0.08)
Feb. 2015	42.04 (0.98)	39.31 (0.64)	41.07 (1.87)	37.42 (1.84)	1.12 (0.21)	0.89 (0.07)	1.07 (0.16)	0.76 ((0.04)
Mar. 2015	41.33 (1.88)	37.66 (0.73)	40.39 (1.22)	37.09 (0.42)	1.14 (0.21)	0.84 (0.07)	1.01 (0.10)	0.78 (0.03)
Apr. 2015	41.55 (0.67)	38.25 (0.45)	40.86 (1.18)	36.10 (0.47)	1.11 (0.05)	0.87 (0.04)	1.06 (0.11)	0.76 (0.03)
May 2015	42.92 (1.62)	38.50 (0.76)	42.88 (0.65)	37.50 (0.50)	1.17 (0.12)	0.86 (0.05)	1.23 (0.05)	0.88 (0.04)
June 2015	47.17 (1.18)	41.14 (1.07)	46.10 (0.62)	41.18 (0.40)	1.66 (0.15)	1.03 (0.09)	1.62 (0.08)	1.11 (0.04)
July 2015	46.66 (0.75)	38.98 (0.50)	46.42 (0.81)	40.66 (0.70)	1.70 (0.10)	0.88 (0.45)	1.61 (0.11)	1.07 (0.08)
Aug. 2015	48.61 (1.09)	43.37 (1.97)	44.08 (1.96)	42.59 (0.60)	1.84 (0.13)	1.30 (0.18)	1.38 (0.19)	1.21 (0.05)
Mean	**43.66**	**39.19**	**41.62**	**38.53**	**1.30**	**0.93**	**1.12**	**0.88**

Monthly GSI values for each species can be found in Table 2 and Figures 1 and 2. Female and male *E. duryi* and female *E. simoterum* had peak GSI values in March. Male *E. simoterum* had peak GSI values in April. When looking at the monthly average female GSI values (Figure 1), they are almost identical. Female GSI values show that reproductive capacity peaks in March, April, and May with a slow buildup beginning in December. The peak month for both species is March (Figure 1). As seen in Figure 2, there are slight differences in the average male GSI values between species with the values for *E. simoterum* higher most months, with no significant differences.

Table 2. Mean GSI values of male and female *Etheostoma duryi* and *E. simoterum* over a 12-month period from September 2014 to August 2015, with number (n) examined in parentheses.

	Etheostoma duryi		*Etheostoma simoterum*	
Month	Female Mean GSI	Male Mean GSI	Female Mean GSI	Male Mean GSI
September, 2014	0.57 (n = 10)	0.04 (n = 11)	0.51 (n = 27)	0.03 (n = 31)
October, 2014	0.80 (n = 8)	0.10 (n = 7)	0.69 (n = 44)	0.06 (n = 22)
November, 2014	1.39 (n = 12)	0.20 (n = 15)	1.39 (n = 61)	0.16 (n = 19)
December, 2014	2.43 (n = 6)	0.32 (n = 5)	2.51 (n = 12)	0.25 (n = 4)
January, 2015	3.03 (n = 15)	0.56 (n = 17)	3.00 (n = 22)	0.69 (n = 31)
February, 2015	4.34 (n = 19)	0.77 (n = 13)	4.90 (n = 29)	0.89 (n = 11)
March, 2015	9.21 (n = 25)	1.06 (n = 10)	9.02 (n = 48)	1.21 (n = 15)
April, 2015	8.24 (n = 23)	0.87 (n = 6)	8.48 (n = 48)	1.50 (n = 8)
May, 2015	7.28 (n = 17)	0.70 (n = 7)	7.23 (n = 52)	0.56 (n = 18)
June, 2015	0.39 (n = 8)	0.02 (n = 8)	0.44 (n = 56)	0.04 (n = 23)
July, 2015	0.35 (n = 10)	0.02 (n = 6)	0.48 (n = 25)	0.07 (n = 10)
August, 2015	0.36 (n = 5)	0.02 (n = 5)	0.40 (n = 21)	0.04 (n = 8)

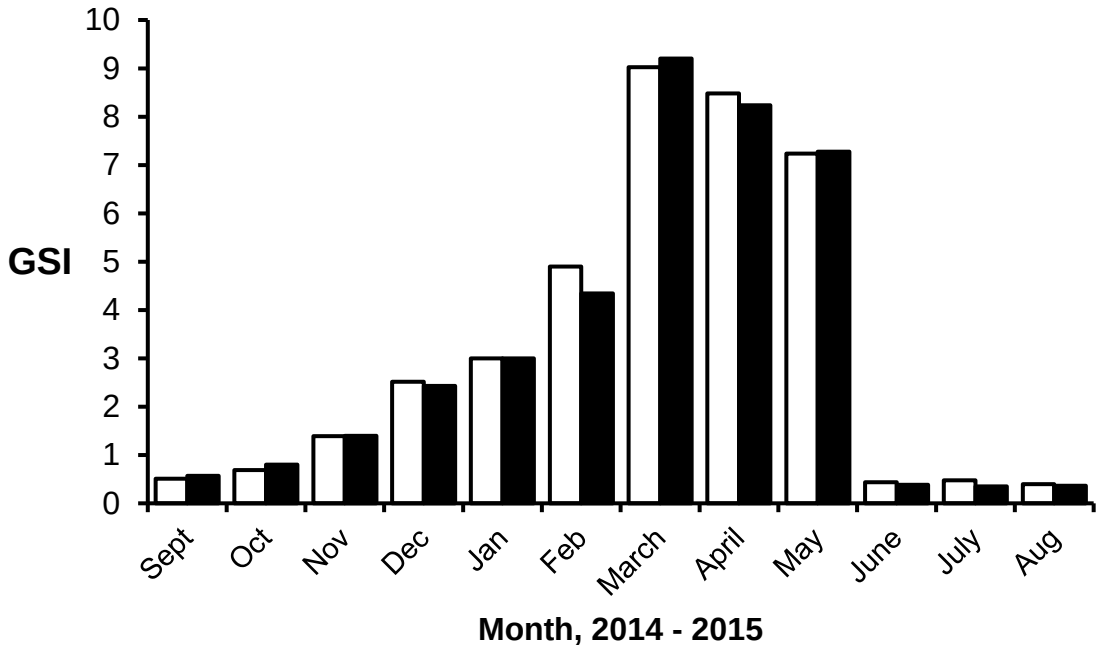

Figure 1. Mean monthly female GSI values for *Etheostoma duryi* and *E. simoterum* over a 12-month period from September 2014 to August 2015. *Etheostoma duryi* is represented by black bars, *E. simoterum* is represented by white bars.

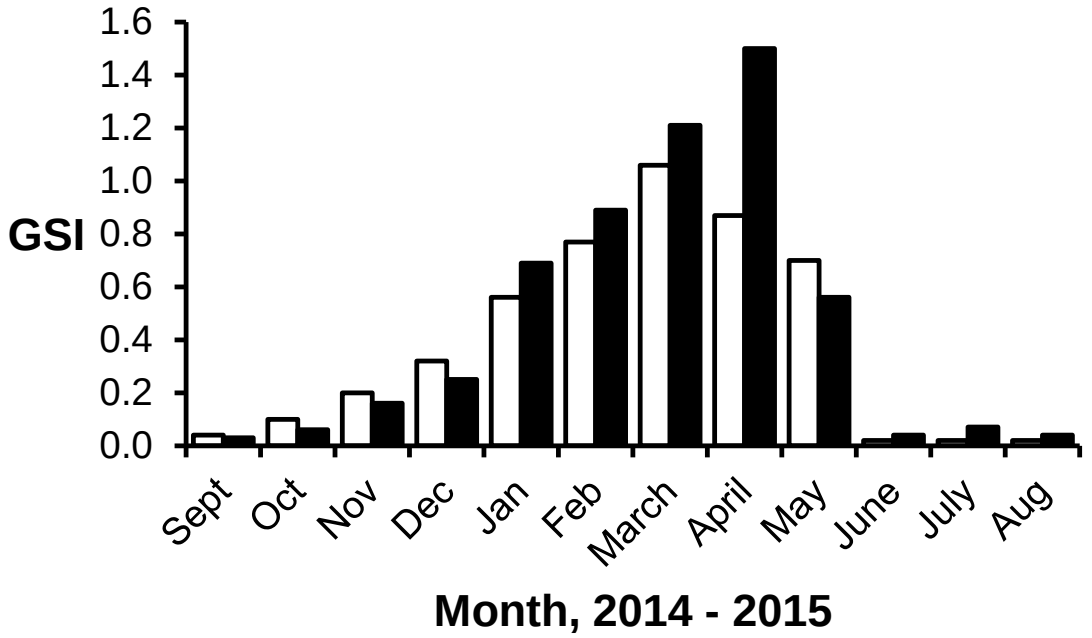

Figure 2. Mean monthly male GSI values for *Etheostoma duryi* and *E. simoterum* over a 12-month period from September 2014 to August 2015. *Etheostoma duryi* is represented by black bars, *E. simoterum* is represented by white bars.

A total of 132 *E. duryi* females and 175 *E. simoterum* females were examined. Mean oocyte counts for each species climbed steadily through February, when they peaked, decreased steadily through May, and declined sharply in June (Figure 3). Oocyte counts for *E. duryi* were noticeably higher than those of *E. simoterum* in January through April. Figure 4 shows that mean monthly clutch values for each species increased sharply in February and dropped to near zero in June. Monthly mean clutch size was larger for *E. duryi* February through May. The largest mean clutch value for *E. duryi* was in April, while peak mean clutch value for *E. simoterum* was in May.

Repeated measures two-way ANOVA was used to test for significant differences separately in mean oocyte and mean clutch counts between the two species for the months of January through May. In this test design Factor A, the vertical labels, was one species or the other. Factor B, the repeated measures, in both instances tested each of the five months for either oocyte or clutch counts of 15 females from each species. The two species were found to be significantly different in mean oocyte counts ($F[1, 4] = 6.6$, $p = 0.003$) meaning that *E. duryi* females carried more oocytes. The two species were also found to be significantly different in mean clutch counts ($F[1, 4] = 6.8$, $p = 0.014$) meaning that *E. duryi* females carried larger clutches.

To test the monthly relationship in both species between female GSI and clutch size from February through May, ANCOVA was performed with GSI as the covariate and clutch size as the dependent variable. The monthly sample size for both species was 15 except for February for *E. simoterum, which* had 14 individuals. For *E. duryi,* the test for homogeneity of regressions rejected homogeneity ($F[3, 55] = 3.40$, $p = 0.02$) and no difference was found between the adjusted means ($F[3, 55] = 2.67$, $p = 0.06$). For *E. simoterum*, the test for homogeneity of regressions did not reject homogeneity ($F[3, 54] = 0.91$) and the adjusted means are significantly different ($F[3, 54] = 8.65$, $p < 0.001$). The heterogeneity found among the four monthly samples of *E. simoterum* means that each month's data can be described with a unique regression line, with distinct slope and intercept. These results suggest different spawning patterns in the two species, with *E. duryi* spawning at a steady rate over the four months and *E. simoterum* varying from month to month.

The diameter (mm) of 5 oocytes of each stage in five separate specimens per month was measured for the months of February through May. Oocytes of each of the five stages were larger in diameter in *E. simoterum* than in *E. duryi* (Figure 5). A repeated measures ANOVA was used to test for significant difference in oocyte diameter between the two species for each of the five developmental stages. Each test had the two species as Factor A, and Factor B was the five measurements from five specimens monthly from February through May. A significant difference was found between the species for each developmental stage as follows: Stage 1 ($F[1, 3] = 165.6$, $p < 0.001$); Stage 2 ($F[1, 3] = 212.9$, $p < 0.001$); Stage 3 ($F[1, 3] = 98.1$, $p < 0.001$); and Stage 4 ($F[1, 3] = 27.2$, $p < 0.001$). The oocytes of *E. simoterum* were significantly larger than those of *E. duryi* at each developmental stage.

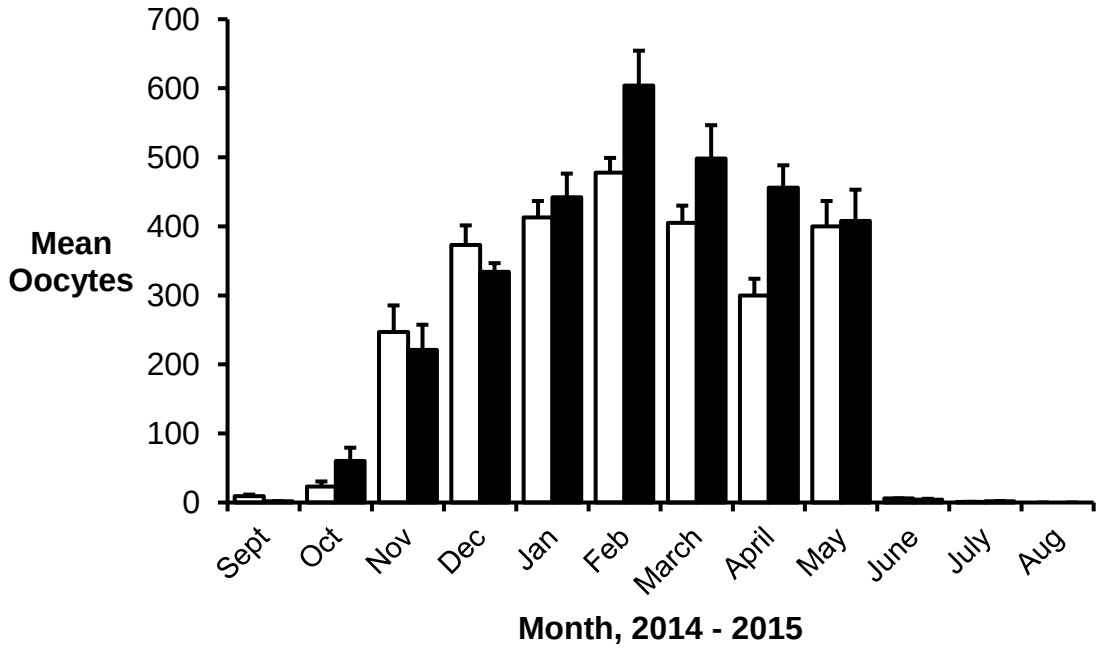

Figure 3. Mean monthly oocyte counts for *Etheostoma duryi* and *E. simoterum* calculated using 15 specimens for each month (except in months where less than 15 specimens were collected) over a 12-month period from September 2014 to August 2015. *Etheostoma duryi* is represented by black bars, *E. simoterum* is represented by white bars. Error bars = 1 SE.

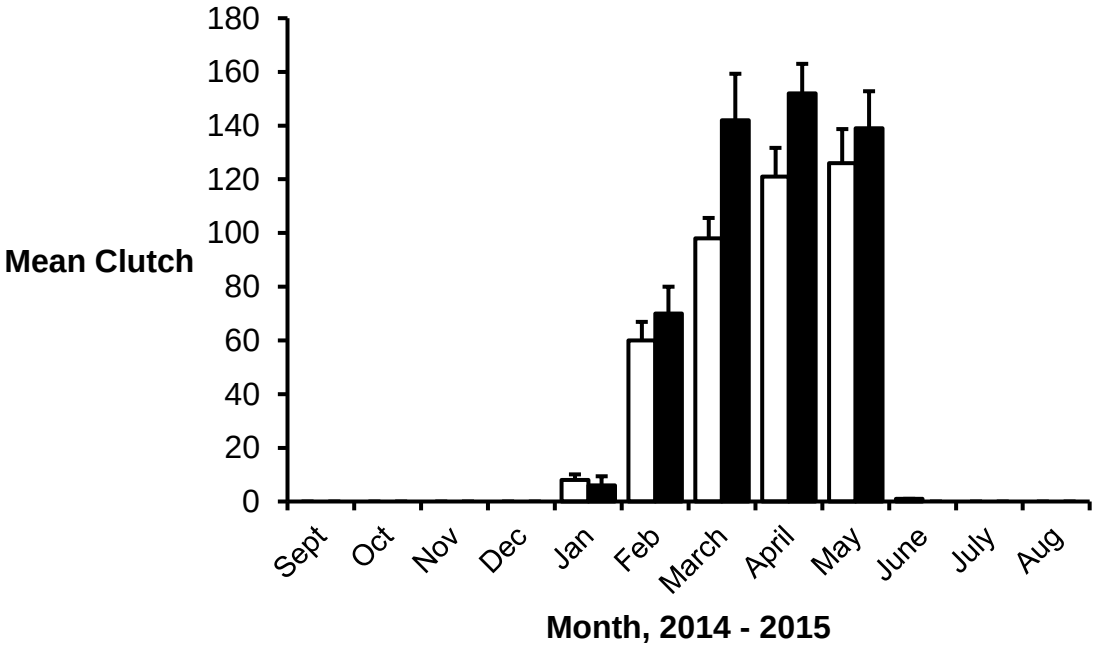

Figure 4. Mean monthly clutch sizes for *Etheostoma duryi* and *E. simoterum* calculated using 15 specimens for each month (except in months where less than 15 specimens were collected) over a 12-month period from September 2014 to August 2015. *Etheostoma duryi* is represented by black bars, *E. simoterum* is represented by white bars. Error bars = 1 SE.

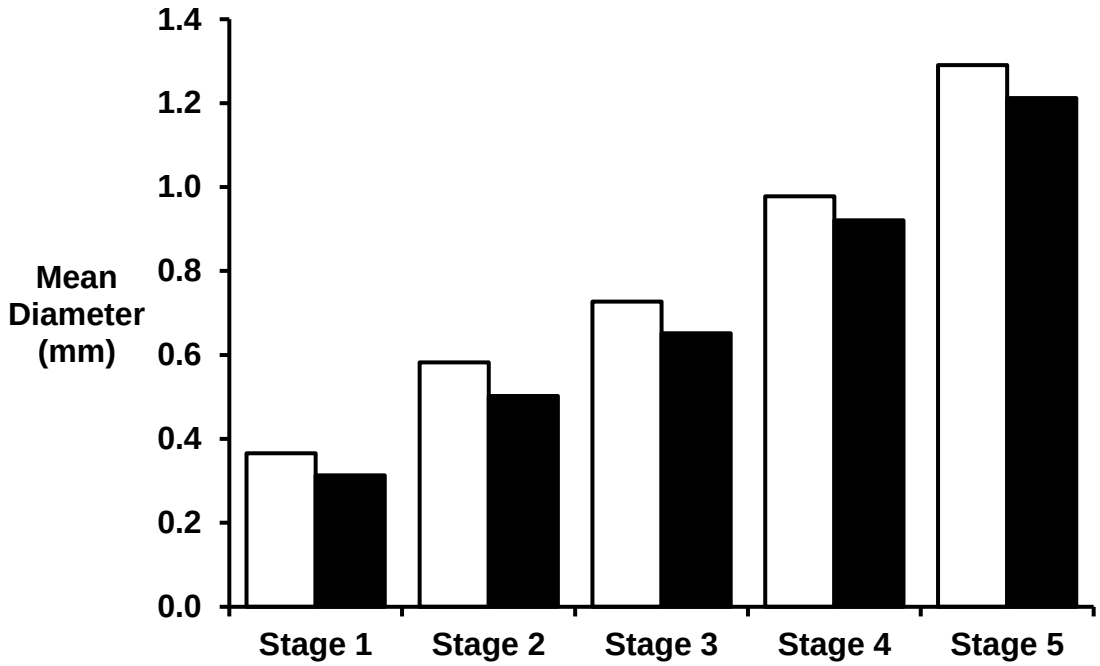

Figure 5. Mean oocyte diameter for *Etheostoma duryi* and *E. simoterum* calculated by measuring the diameter (mm) of 5 oocytes of each stage in 5 specimens for reproductive months (February–May, 2014 and 2015). *Etheostoma duryi* is represented by black bars, *E. simoterum* is represented by white bars.

DISCUSSION

Like many other darter species, *E. duryi* and *E. simoterum* co-occur in many locations. There is a difference in the peak of male GSI values; the peak occurs in March for *E. duryi* and in April for *E. simoterum*. Female GSI in both species peaks in May. We found that GSI values were very similar for the two species, and both reached a reproductive peak at the same time in March and April. However, clutch size, size of oocytes, and numbers of oocytes differed during the reproductive season.

An important finding is that *E. duryi* produces a larger number of smaller oocytes than *E. simoterum.* With the production of more oocytes, *E. duryi* also has a larger clutch. That *E. duryi* produce more oocytes raises questions about possible early life history differences between the two species. These differences may reflect that *E. simoterum* is better adapted to faster riffles, and its reproductive strategy compared to *E. duryi* is to cope with more challenging small stream habitats. To answer these questions requires field observations beyond the original scope of this research.

The larger mean monthly clutch size of *E. duryi* is not the only difference between the two species. There is also a significant difference in pattern of the relationship between GSI and clutch size. Testing the relationship between mean GSI and clutch size showed that *E. simoterum* had varying mean clutch size between the four months examined, but with a homogeneity of relationship between GSI and clutch size. This is the opposite of *E. duryi*, with no significant

differences found in mean clutch size but heterogeneous relationships between GSI and clutch size. These are subtle differences, since superficial examination of monthly mean GSI and clutch size over the four months suggests little difference between the two species. The ANCOVA results suggest that *E. duryi* produces clutches in a steady, maybe predictable fashion while *E. simoterum* clutch production varies over the reproductive season in response to one or more factors.

Etheostoma duryi may require more resources to produce a larger clutch size. Based on observations during dissection, *E. simoterum* possesses more fatty tissue than *E. duryi*, especially during breeding season. This suggests that *E. simoterum* allocates more resources to maintaining body fat while *E. duryi* dedicates these resources more immediately to reproduction. This observation could also indicate a difference in diet. In their comprehensive study of the ecological morphology of darters, Carlson and Wainwright (2010) found that what they called the "*E. blennioides* clade", now known as *Simoperca*, have strong bite force with their small, downturned mouths but they lack speed and agility in their bites. This arrangement is well suited for grazing small benthic prey items and is especially true of the *E. simoterum* complex, enabling an ecomorphic foraging strategy called "the manipulator." *Etheostoma simoterum* has a more downturned mouth than does *E. duryi*. Perhaps this means that *E. simoterum* feeds primarily on the top of rocks and other structures while *E. duryi* feeds primarily on the sides of these structures with implications for quantity and quality of food.

More females than males of both species were caught during breeding season. Because neither of these species participates in nest guarding or any type of parental care after the release of eggs and sperm, the higher number of females caught cannot be attributed to females staying with the eggs that they deposited. Thompson and Stallsmith (2015) observed differences in habitat use between males and females in *E. duryi* during the pre-spawn period. Females were more commonly found in areas where there was decreased water flow and lower boulder content. Such habitat coincidentally is often easier for researchers to capture fish by seining; thus, observed female-biased sex ratios may reflect differences in habitat use among sexes rather than an actual difference in sex ratio during the breeding season.

Similarly, more female *E. simoterum* were caught in all months but one, not only during breeding season. Thompson and Stallsmith (2015) observed a difference in habitat utilization among males and females of this species during the post-spawn period. However, this does not explain why more females were caught nearly every month during this research. Page and Mayden (1981) also observed female-biased sex ratios in their study of the life history of *E. simoterum*. They found that females were 1.8 times as abundant as males and that more females survived to a second year of spawning than males. Similar patterns have been found in other *Simoperca* darter species. Johnston and Haag (1996) found a 1.5:1 female skew in *E. raneyi,* and found that few males survived to a second breeding season. Khudamrongsawat et al. (2005) studied *E. chermocki* found a statistically significant female skew of 1.2:1. Thus, female skew appears to be common in *Simoperca* darter species.

A possible explanation for these findings would be that male-male competition leads to male attrition. Recent work with sexually dimorphic *E. spectabile* and *E. caeruleum* darters by Moran and Fuller (2018) strongly suggests that male coloration and behavior in darters are

driven by male-male interactions rather than by female choice as long thought. Such processes sharpen species differences in sympatry, prompting intraspecific rather than interspecific male conflict. A sharpening of male-male conflict could lead to higher rates of male attrition, resulting in the strong female skew we found as did Page and Mayden (1981), Johnston and Haag (1996), and Khudamrongsawat et al. (2005). Perhaps further observation of both species in nature could help to begin to answer this question of possible male-male competition. It is important to consider the effect that the answer to this question has on the reproductive strategies of both *E. duryi* and *E. simoterum*.

LITERATURE CITED

Boschung, H. T., & Mayden, R. L. (2004). Fishes of Alabama. Smithsonian Books. Washington, DC.

Carlson, R. L., & Wainwright, P. C. (2010). The ecological morphology of darter fishes (Percidae: Etheostomatinae). *Biological Journal of the Linnean Society*, 100, 30–45.

Daniel's XL Toolbox add-in for Excel, version 7.3.4, by Daniel Kraus, Würzburg, Germany (www.xltoolbox.net).

Gotelli, N. J., & Ellison, A. M. (2013). *A Primer of Ecological Statistics, 2nd ed.* Sunderland, MA: Sinauer Associates.

Heins, D. C. (2001). Variation in clutch size and ovum size of the snubnose darter, *Etheostoma simoterum* (Cope), from two populations in Tennessee. *American Midland Naturalist*, 45, 74–79.

Heins, D. C., & Rabito, F. G. Jr. (1986). Spawning performance in North American minnows: direct evidence of the occurrence of multiple clutches in the genus Notropis. *Journal of Fish Biology*, 8**,** 343–357.

Johnston, C. E., & Haag, W. R. (1996). Life History of the Yazoo Darter (Percidae: *Etheostoma raneyi*), a species endemic to north-central Mississippi. *Tulane Studies in Zoology and Botany*, 30, 47–60.

Khudamrongsawat, J., Arrington, A., Kuhajda, B. R. & Rypel, A. L. (2005) Life History of the Endangered Vermilion Darter (*Etheostoma chermocki*) Endemic to the Black Warrior River System, Alabama. *Journal of Freshwater Ecology*, 20, 469–477.

Million, K. M., Tarver, C. L., Hipe, S. & Stallsmith, B. W. (2017). Does infection by the monogenoidean gill parasite *Aethycteron moorei* affect reproductive ecology of the darter *Etheostoma flabellare* in Mill Creek, Tennessee? *Copeia*, 105, 75–81.

Moran, R. L., & Fuller, R. C. (2018). Agonistic character displacement of genetically based male colour patterns across darters. *Proceedings of the Royal Society B*, 285 20181248.

Near, T. J., Bossu, C. M., Bradhurd, G. S., Carlson, R. L., Harrington, R. C., Hollingsworth, P. R., Keck, B. P., & Etnier, D. A. (2011) Phylogeny and temporal diversification of darters (Percidae: Etheostomatinae). *Systematic Biology*, 60, 565–95.

Near, T. J., France, E. D., Keck, B. P., & Harrington, R. C. (2016). Systematics and taxonomy of the snubnose darter, *Etheostoma simoterum* (Cope). *Bulletin of the Peabody Museum of Natural History*, *57*(2), 127–145.

Page, L.M., & Mayden, R. L. (1981). The life history of the Tennessee snubnose darter, *Etheostoma simoterum* in Brush Creek, Tennessee. *Biological Notes of the Illinois Natural History Survey*, 117, 1–11.

Stallsmith, B., & Bedingfield, R. (2015). Reproductive development in the black darter (*Etheostoma duryi*). *Journal of the Alabama Academy of Science*, 86, 15–28.

Tarver, C., & Tarver, R. (2014). EggHelper. Dark 30 Technologies, LLC. Available from https://www. dark30technologies.com.

Thompson, B., & Stallsmith, B. (2015). Microhabitat partitioning of an assemblage of darter species within two tributaries of the Tennessee River in Alabama. *River Research and Applications*, 1–10.

VassarStats: Website for Statistical Computation. 2020. http://vassarstats.net/.