12-8-2016

Reproductive Timing of the Striped Shiner (Lythrurus fasciolaris) in Northern Alabama

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Reproductive timing of the Scarlet Shiner (*Lythrurus fasciolaris*) in Northern Alabama

by

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An Honors Capstone

submitted in partial fulfillment of the requirements

for the Honors Diploma

to

The Honors College

of

The University of Alabama in Huntsville

12/5/16

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Abstract

The aim of this project was to determine the peak reproductive period of the freshwater fish, *Lythrurus fasciolaris,* (Scarlet Shiners) in the Flint River of Northern Alabama. This was done by taking monthly samples of approximately 30 adults from the Flint River from September 2014 through August 2015. The fish collected were catalogued, measured, and their gonads were excised. The excised gonads were measured and masses were compared to the mass of the fish to calculate their gonadosomatic index (GSI). Oocytes were removed from the ovaries and classified into stages. The oocytes were classified into four distinct stages based on development, ranging from early maturation to ripe. The quantities of oocytes in the various stages and GSI were used to determine the reproductive potential of the individuals and their clutch sizes. It was found that the months with the highest GSI and clutch sizes were April through July, especially April with the largest average clutch size that was statistically significant. This was found to be the period of peak reproductive activity and fecundity.

Introduction

The Flint River is one of three rivers located in the Apalachicola-Chattahooche-Flint (ACF) River Basin. These three rivers provide a large amount of the freshwater that moves toward the Gulf of Mexico. The ACF River Basin is home to over 122 species of fish from 23 families. Amongst these are 33 members of the Cyprinidae family, which includes *Lythrurus fasciolaris*, the Scarlet Shiner. There are also 20 members of Centrachidae, the sunfish family, as well as many species introduced by humans including: rainbow and black trout, white and flathead catfish, black bullhead, goldfish, carp, and rough and red shiners (Couch et al. 1996).

The Scarlet Shiner is a small freshwater fish. Fully grown adults can reach up to 90 mm in length and are thought to live up to three years. Within the rivers they often inhabit calmer pools and small to medium streams (Boschung and Mayden 2004). Not much is known about their reproductive schedules, but their spawning may be associated with some species from the genera *Lepomis* (sunfish) and *Nocomis* (chub) from the months of May to August. There is also evidence that the Scarlet Shiner crossbreeds with the Redfin Shiner (*Lythrurus umbratilis*) in some drainages of the Ohio River Basin (Hopkins and Eisenhour 2008).

Scarlet Shiners use ornamentation to attract their mates, both through tubercles around their mouths and bright red coloration of the males during mating season. This coloration is a signal of the male being in peak physical condition through the consumption, digestion, and display of carotenoids. This display is triggered through the androgen 11-ketotestosterone, which can also cause an increase in size and aggression when present (Schade and Stallsmith 2012). Female Scarlet Shiners amass oocytes in their ovaries that are typical of aquatic oviparous species. These oocytes contain many products used to sustain the developing fish until it is fully

self-sufficient, such as proteins (both growth and transcription factors), lipids, vitamins, and hormones. These proteins are stored in the yolk of the mother's eggs and oocyte growth occurs by the uptake of plasma egg yolk precursor proteins by the mother. By the end of the vitellogenesis phase, the oocytes and gonads will be completely developed and can take up around 20% of the fish's total mass (Babin et al. 2007).

This study held the key objective of determining the schedule of reproductive viability of Scarlet Shiners and how it coordinates with other species in its surrounding habitats. This was done by examining the GSI and maturation of oocytes (in method of Heins and Rabito 1986). One similar study was done previously by Stallsmith and Taylor with results similar to the behaviors observed by Hopkins and Eisenhower, with peak activity being from April to August (Stallsmith and Taylor 2013).

Materials and Methods

Collection Site

The fish were randomly collected from the Flint River in Madison County Alabama between the months of September, 2014, to August, 2015. The collections were made at a site along the river at Oscar Patterson Road (34° 52" 50' N, 86° 28" 50' W). There was no bias in the collections on the basis of size or sex. This site was selected due to it being representative of typical habitat preferred by Scarlet Shiners. It has medium-sized streams with a riverbed composed of a variation of rock, pebble, and gravel substrate and medium water flow (Boschung and Mayden 2004).

Fish Capture

The Scarlet Shiners were collected using a combination of kick-seine and cast net techniques. A variation of mesh sizes were used. The seine net was 3.5 m long and 1.2 m deep, with 3 mm mesh. The cast net had a diameter of 2.3 m, with a mesh size of .75mm. Around 30 specimen were collected on average each month, with the number varying depending on availability depending on weather and seasonality. The captured specimens were contained in a metal bucket until leaving the collection site. Once removed from the bucket the fish were euthanized in either clove oil or tricaine methanesulfonate (MS-222). Once they were returned to the lab the fish were placed into glass collection jars and fixed in 10% phosphate buffered formalin.

Data Collection

Each fish was given an ID number based on its species, the month it was collected, and the order it was catalogued. The standard length and weight was recorded for each fish. Standard length was measured from the tip of the jaw to the caudal peduncle for each specimen using a digital caliper (Fisher Scientific). The standard length was measured to the nearest hundredth of a millimeter. Each fish was dried and weighed on an Ohaus® Explorer balance. Weight was measured to the nearest hundredth of a gram.

Fish that were over 41 mm in length were deemed mature adults, and the sex of the fish was recorded for these specimens. These fish had mature gonadal tissue, which was used to determine the gonadosomatic index (GSI) of the fish. This statistic is calculated by finding the percentage of the fish's gross mass that its gonadal mass is. It was calculated using the formula: GSI = (gonadal mass / (total mass – gonadal mass)) x 100. If a fish was considered juvenile it was not dissected or sexed.

Reproductive Evaluation

As stated above, the specimen's status as a juvenile or adult was determined based on length. Any fish with a standard length of than 41 mm or more were considered a reproductively mature adult. This classification was justified by observations that specimens smaller than this did not have visible gonadal tissue. Adult specimens had their gonadal tissue removed and preserved separately from the rest of the body. Images of intact gonadal tissues, as well as separated oocytes for the females, were captured using an Olympus SZX7 dissecting microscope with an Olympus DP72 camera. These images were analyzed to determine oocyte maturation

using the Cellsens Standard software that is distributed with this camera. The images were captured at a magnification of 8.4X (1.6X x 4.0x) and saved as .tiff files.

To capture images of the female oocytes, ovaries were teased apart. The individual oocytes were photographed and the images viewed inside EggHelper software to count the oocytes. In the images the oocytes were arranged in a single layer, for the purpose of imaging and using EggHelper. If all of the oocytes could not be captured in one image, multiple images were taken. Some ovaries required as many as five images. Oocytes were then classified using size and color as indicators of maturation. This classification scheme excluded any latent oocytes present in the ovaries. Stage 1 was deemed the early maturing stage and were the smallest eggs that had an almost clear coloration. This egg type had not yet fully matured as a functionally viable egg, thus the clear color and small size. Stage 2 was determined to be the late maturing stage and were similar in size to Stage 1, but had started developing the opaque yellow coloration of a mature oocyte. Stage 3 was determined to be the mature stage and were characterized by a larger size than either Stage 1 or Stage 2, but maintained the yellow coloration found in Stage 2. Stage 4 was deemed the ripe stage and was characterized by the largest size of any of the stages and the presence of clear ring along the outside of the oocyte or completely clear coloration. Ripe oocytes are not present in the fish long, as they are quickly released upon reaching this stage of development. Therefore, this stage of oocyte is not often found in collected specimens. The oocytes were counted according to their classifications in these stages. Clutch size was determined by combining the number of eggs counted as Stage 3 and Stage 4.

Data Analysis

One-way ANOVA was used to test for any significant relationships between monthly average clutch sizes and GSI values. Monthly averages of GSI and clutch size were found, along with standard error (SE) as a measure of variance. In addition, A Tukey Post Hoc test was run to determine any patterns and significant differences between the data collected for each month.

Results

Over the course of the 12-month study a total of 425 specimens were collected: 91 females, 97 males, and 237 juveniles. Collection sizes varied in number of specimen collected per month, ranging from 5 to 65. At least one adult male and female were collected for each month. Juveniles were not used for any of the statistics on reproductive development, due to the lack of fully developed gonads.

Reproductive Development

The system used to categorize the oocytes was based on the schema laid out by Holmes et al. (2010). There were females with oocyte containing ovaries seven out of the twelve months of the study, with five of those months having ovaries containing developed, reproductively viable oocytes. The oocytes were categorized into four different stages based off of their developmental progress, which was characterized by color and size (Figure 1). Any stage 3 or stage 4 oocytes were an indicator that the ova were mature, which classified the female as either late maturing or mature.

When considering females with ovaries containing oocytes, the number of oocytes per fish ranged from 8 to 991. The average total number of oocytes per month ranged from 8 in February of 2015, to 562 in April of 2015. Within the months containing mature oocytes, average clutch sizes ranged from 105 oocytes in August of 2015, to 333 oocytes in April of 2015 (Figure 4). A one-way ANOVA was performed to determine if there was a significant difference in average monthly clutch sizes. The ANOVA results suggested there was a difference, with a

value of P=.0227. Due to this, a post-hoc Tukey HSD test was preformed to do pairwise comparisons between each month. The results showed there was a significant difference between the months of April 2015 and August 2015, with a value of P<.01.

Monthly average GSI values are shown in Figure 2. A one-way ANOVA was performed on the monthly GSI average to determine if there was any significant difference between the monthly averages. The initial ANOVA came back with female GSI having a value of P=0.0011 and male GSI having a value of P=0.17. This suggested that one or more of the months are significantly different than the others for the females, but there was no significant difference between the months for the males. Because of this a post-hoc Tukey HSD test was run on the data for the females to determine which months were significantly different. The comparison results showed that the average GSI for May had a value of P<.01 when compared to any other month, but all other months did not have significant values for P.

The average number of oocytes per fish per month, as well as per stage, is shown in Figure 3. From April until August there were high numbers of Stage 3 oocytes present. Stage 4 oocytes were only present from April until July, and always accounted for less than 8% of total oocytes present.

Discussion

GSI Measurements and Reproductive Season

The analysis of the GSI data on the collected Scarlet Shiners also supported the hypothesis that April through August is the peak spawning season for Scarlet Shiners. Female Scarlet Shiners had elevated GSI from April through August of 2015, with August beginning the decline back towards non-reproductive GSI values. In May 2015 the females showed the highest peak in elevated GSI. The males, however maintain relatively constant GSI values throughout the year, with no pattern present to explain the slight variations.

Based on these results, the Scarlet Shiner is a late spawner compared to other cyprinid species present in the Flint River. The Blotched Chub (*Erimystax insignis*) has its spawning season from March to May (Stallsmith and Allen 2014) and the Silver Shiner (*Notropis photogenis*) has its spawning season from February to April (Hodgskins et al. 2016). The Whitetail Shiner (*Cyprinella galctura*) has a similar spawning season to the Scarlet Shiner, with spawning behavior being observed from May to August. This similarity can be credited to similar reproductive strategies focusing on sexual dimorphism and selection based on large "bourgeois" males competing for females and defending territory. This reproductive strategy tends to take place in warmer waters, which are more desirable to the females and more sought out by the males. Most other cyprinid species produce more oocytes per females than either the Scarlet or Whitetail Shiners, but their oocytes tend to be smaller. This may be due to the fact that other species such as the Blotched and Silver Shiners are broadcast spawners.

The comparatively long breeding season of Scarlet Shiners, which is five months long compared to the three month breeding season of other similar cyprinids, could be attributed to

females producing multiple clutches of fewer oocytes. This is also supported by the lower number of oocytes present in the females.

Oocyte Development

The purpose of this study was to learn the reproductive schedule of *Lythrurus fasciolaris*, the Scarlet Shiner. Other similar studies have been done previous concerning other species (Holmes et al. 2010, Durham and Wilde 2014), in which the same methods of examining oocyte numbers and maturity and ovarian condition were used to determine reproductive seasons. However, despite commonalities in approach, no universal method has been developed to determine fecundity due to variations among species (Murua et al. 2013).

The results of this study showed that there was significant difference in the average clutch size present in April (considered the start of spawning season). It is not uncommon during spawning season for the ovaries to occupy up anywhere from ¼ to ¾ of the body cavity depending on species (Murua et al. 2013). The Scarlet Shiners demonstrate this with the large presence of mature oocytes found in specimens collected in the peak of spawning season. Because April was the month with a significantly higher average clutch, it appears that April is the most important spawning month for Scarlet Shiners. Stage 1 oocytes were also present leading up to peak spawning season, as well as throughout the season. Stage 4 oocytes were constantly in the lowest percentage of the total oocytes present, and were present April through August of 2015, however in the months of April and August they were present so rarely that the average came to zero. The rarity in presence of oocytes in this stage is due to the fact that once

the oocytes reach this point in development the eggs are quickly shed, making it difficult to collect specimens at the point of development.

There were specimens collected in April, June, and July of 2015 that were mature females, but did not have oocytes present in the ovaries. There are many processes that may account for the absence of ovaries during peak spawning season. The first explanation is that these females could have been temporarily regressed during that particular phase of spawning season. A second explanation would be that the specimens were "mature inactive," meaning that the females are partially mature and began reproduction but aborted the production of viable oocytes without producing any oocytes. This often occurs in individuals who are going through spawning season as reproductively mature adults for the first time. The final explanation is that the females had already shed their eggs for spawning and were in the resting phase when they were collected. This resting stage occurs after the mature stage and ovaries may only contain a few residual oocytes, post-ovulatory follicles (POF) and hydrated oocytes (Murua et al. 2013). Murua's hydrated oocytes is synonymous with Heins and Rabito's (1986) latent oocytes.

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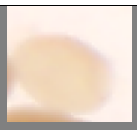
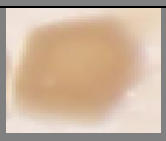
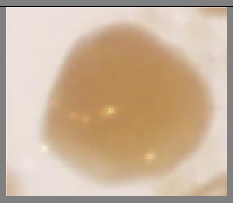
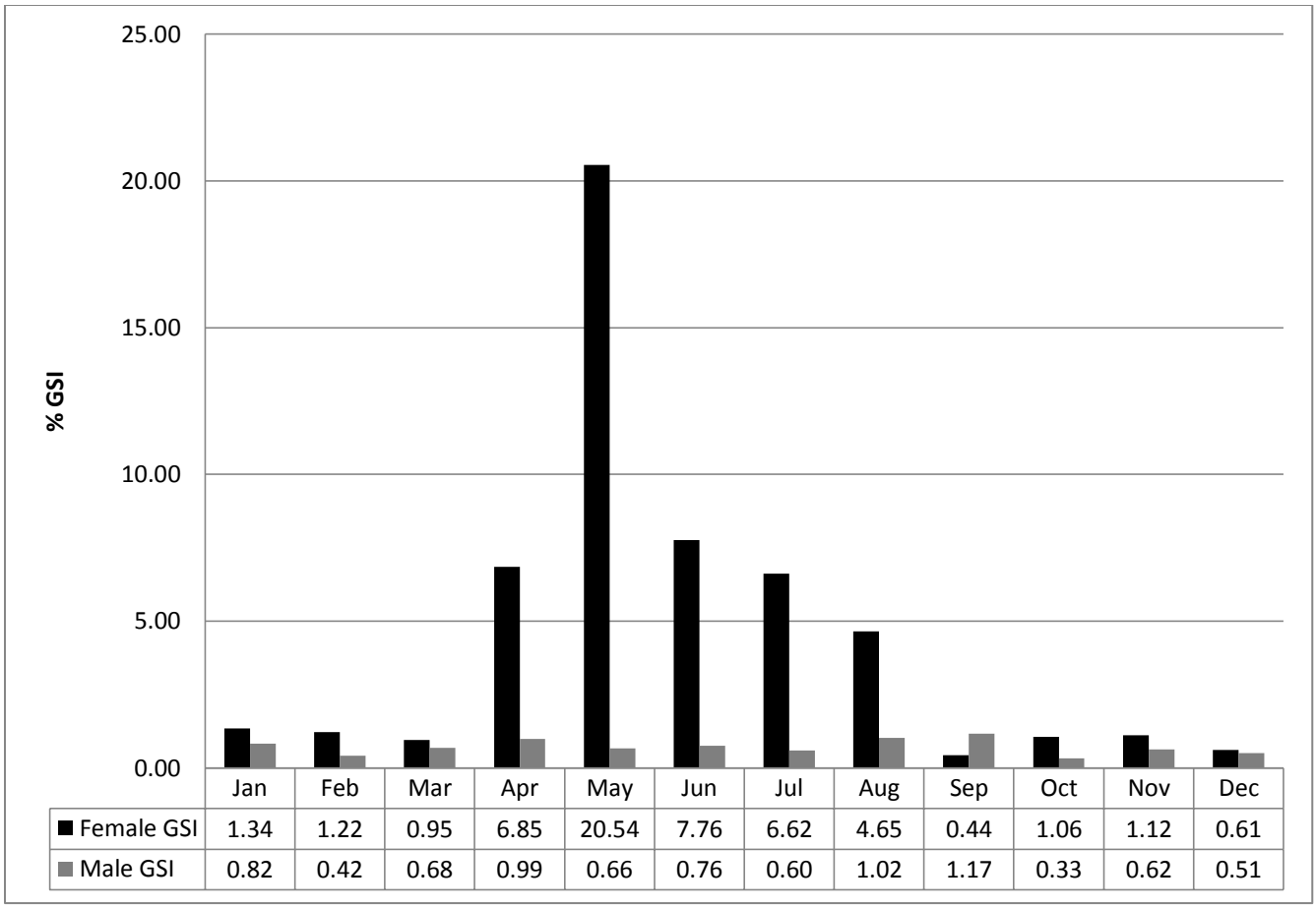
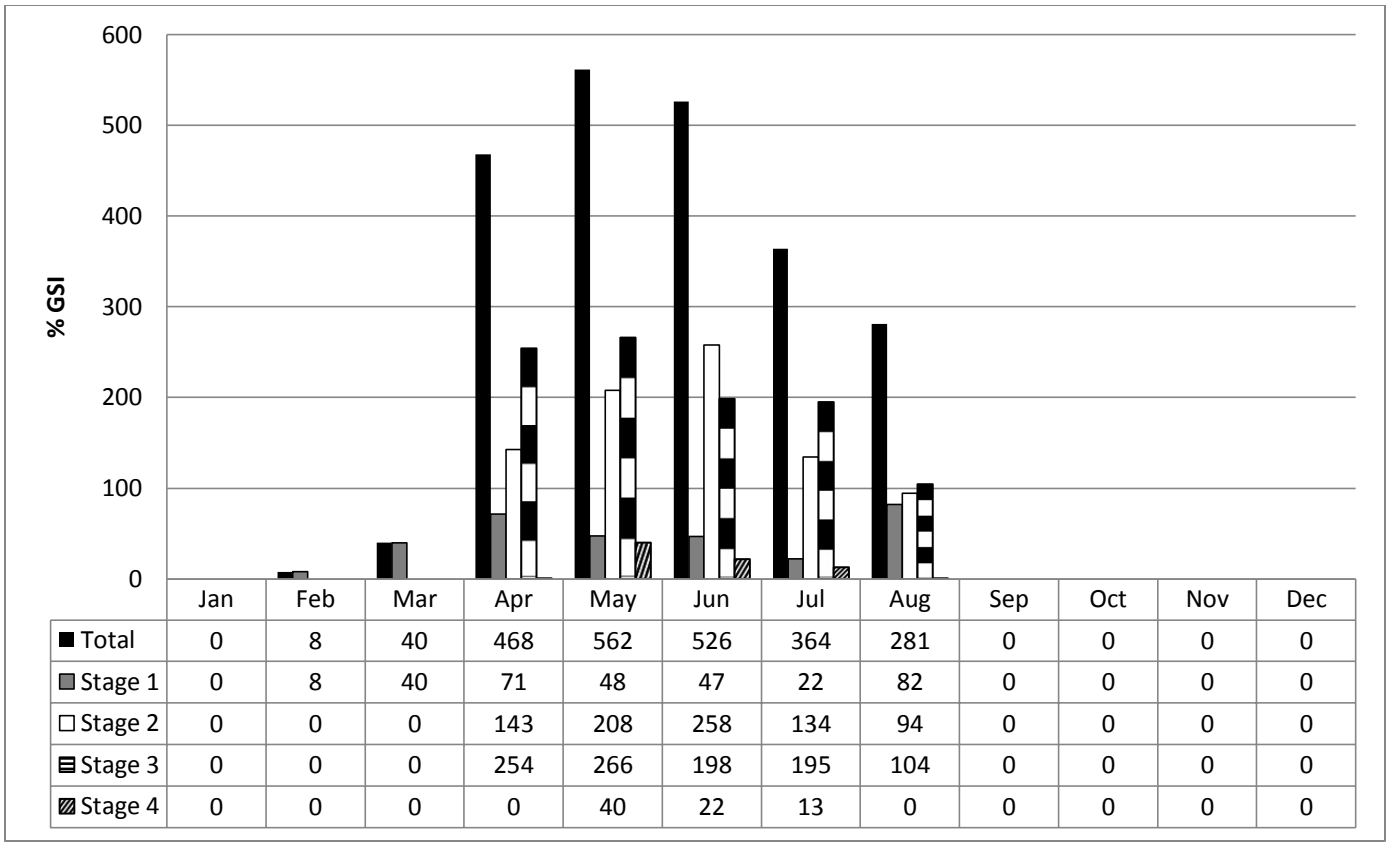
	Stage 1
	Stage 2
	Stage 3
	Stage 4

Figure 1. Images of oocytes at each maturation stage.



	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Female GSI	1.34	1.22	0.95	6.85	20.54	7.76	6.62	4.65	0.44	1.06	1.12	0.61
Male GSI	0.82	0.42	0.68	0.99	0.66	0.76	0.60	1.02	1.17	0.33	0.62	0.51

Figure 2. Monthly average GSI for mature adult specimens is shown for the 12 months from September 2014 to August 2015. 188 specimens were examined for GSI calculations out of the total 425 specimens collected. Peak spawning was shown to be in May based on female GSI.



	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Total	0	8	40	468	562	526	364	281	0	0	0	0
Stage 1	0	8	40	71	48	47	22	82	0	0	0	0
Stage 2	0	0	0	143	208	258	134	94	0	0	0	0
Stage 3	0	0	0	254	266	198	195	104	0	0	0	0
Stage 4	0	0	0	0	40	22	13	0	0	0	0	0

Figure 3. Average total oocytes was found for each month, as well as averages for each of the stages 1-4. Only developing or developed oocytes were categorized or counted.

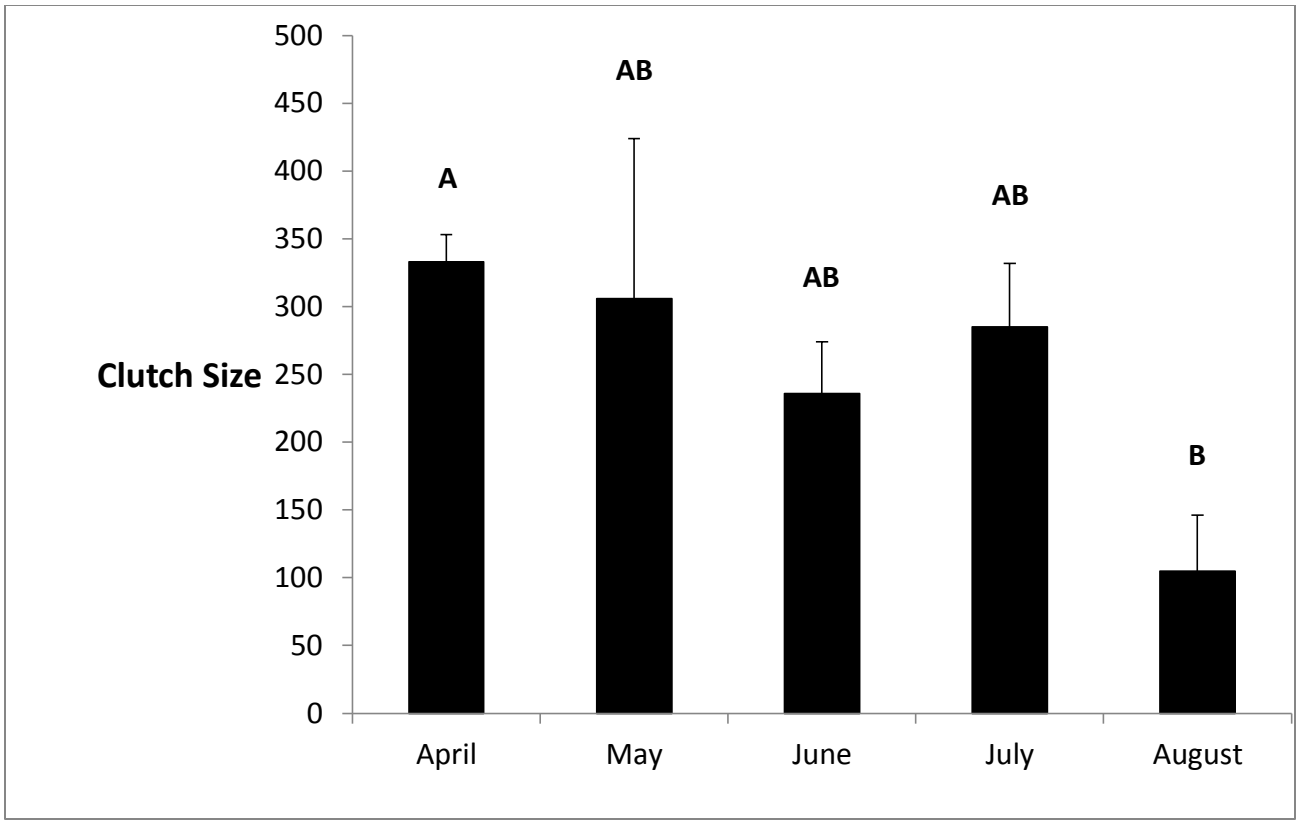


Figure 4. Average clutch size for months during spawning season. April was found to be significantly different from August, but the months in between were not found to be significantly different from either April or August. Error bars are one standard error.