The Reproductive Season of the Highland Stoneroller, *Campostoma spadiceum*, Evidenced by Museum Specimens

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Running Title: Reproductive Season of *Campostoma spadiceum* in Arkansas

Abstract

The Highland Stoneroller (Campostoma spadiceum) was described as a distinct species in 2010. Since then, the only study specific to this species is a survey of distribution, and nothing is known about reproduction. We examined 134 lots including 315 specimens of *C. spadiceum* housed in the Henderson State University collection of fishes to evaluate the timing of reproductive events. We dissected individuals to reveal sex and reproductive status. Females as small as 49 mm total length were yolking eggs, and follicles were in development by October. Ripe (mature) eggs were present in specimens collected from January through May, but were most common in March through May. Several females had oviposited in early March, and most specimens that appeared to have spawned had done so likely in April. Nuptial tubercles appeared on males as early as January and February, but most adult males were fully tuberculated from March through May.

Introduction

The Highland Stoneroller (Campostoma spadiceum) was redescribed as a distinct species (Cashner et al. 2010), soon after cladistic analysis of mitochondrial DNA data (cytochrome b gene) revealed that the population in the Ouachita Mountains region of Arkansas and Oklahoma should be considered a distinct species (Blum et al. 2008). Specimens of all sizes and both sexes present obvious red to red-orange coloration in median fins and usually in paired fins. The coloration lasts year-round and is most intense during the summer, but the coloration is not found in contiguous populations of any other species of *Campostoma* (Cashner et al. 2010).

Since the description, few other studies have dealt with the biology of this species. Some parasites have been documented from Highland Stonerollers, such as Acanthocephalans (McAllister et al. 2016), “black grubs” (McAllister et al. 2013), “white grubs” (McAllister et al. 2014), and leeches (Richardson et al. 2013; 2014). Hodges and Magoullick (2011) followed movement of *C. spadiceum* from riffles to pools during periods of stream drying, and Schanke (2013) studied genetics of subpopulations produced by isolating barriers such as stream drying, waterfalls, and culverts. The only other study specific to this species is a survey of distribution (Tumlison and Robison 2018).

Information concerning reproduction in *C. spadiceum* consists of a few anecdotal observations. Nuptial colors of males peaked in March or April (Cashner et al. 2010), and tuberculated males were observed from March through late April (Tumlison et al. 2017). Mature specimens of both sexes were observed in Clark Co. on 3 March (deemed to be early), and a tuberculated male was collected on 15 November, thought to be long after the breeding season (Tumlison et al. 2017). In Oklahoma, gravid *C. spadiceum* were collected on 13 February (McAllister and Robison 2016). Little is known about sizes of individuals in which reproduction begins, or the peak or duration of oviposition. We sought to provide information about the peak and duration of the reproductive season, and to assess the possibility of multiple spawning in this newly described stoneroller.

Methods

We examined 134 lots including 315 specimens of *C. spadiceum*, housed in the Henderson State University collection of fishes, to evaluate the timing of reproductive events. Individuals had been fixed in 10% formalin prior to washing and transfer to 45% isopropanol for storage. Total length (TL) was measured (mm) to determine at what size individuals became reproductive. We dissected individuals by cutting them open from vent to pectoral fins, then snipping at those points along the left side to create a fold to expose the abdominal cavity. We examined specimens under a
We adapted criteria in Núñez and Duponchelle (2008) and Timms (2017) to classify developmental status of ovaries in preserved museum specimens. Ovarian development was treated as having 5 stages (Fig. 1). Stage 1 ovaries are very thin, small, and contain no oocytes, representing the period between reproductive seasons (Timms 2017). We did not include those ovaries in our analysis. Stage 2 ovaries are small but have small, white follicles in which vitellogenesis is not apparent. For our purposes, we included ovaries containing only undeveloped white follicles and those that were white but had started deposition of lipids in Stage 2. Stage 3 ovaries are larger and contain yolking eggs, making them yellow to orange, but some previtellogenic white eggs also are present. Stage 4 ovaries are large and are dominated by ripe eggs that are deep orange to reddish, but eggs from earlier stages also usually are present. Stage 5 ovaries represent individuals that have spawned, so the ovary is a large hollow flat structure, and a few ova may remain. Recent spawners often have a distended, hollow abdomen likely caused by deposition of the eggs leaving space before food added to the intestine refills the vacated space in the abdominal cavity.

Results and Discussion

Left ovaries usually were larger than right ovaries, particularly noticeable in maturing specimens, which was consistent with observations for another stoneroller, C. oligolepis (Timms 2017). Females as small as 49 mm TL were yolking eggs, and follicles were in development by October. Ripe eggs (Stage 4 ovary) were present in specimens collected from January to May, but were most common in March and April. Females that had oviposited were found in early March, but most specimens that appeared to have spawned had done so likely by May (Fig. 2). Stage 3 ovaries were still present into June, which may indicate multiple spawning. Núñez and Duponchelle (2008) noted that multiple spawners have Stage 3 and 4 ovaries containing a mixture of developmental stages of ova. We noted this condition in almost all of our specimens.

Stage 2 ovaries usually had begun deposition of lipids by January or February, causing the eggs to begin to enlarge, but they remained white. Beginnings of development were seen as early as 31 October, and 2 specimens collected on 21 November also were developing eggs. One specimen of only 49 mm TL was undergoing vitellogenesis, and had yellow eggs.
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developing on 16 February. The average size (TL) of females with Stage 2 ovaries declined over the reproductive season (Table 1), and the smallest specimen with Stage 2 ovaries in January was about the same size as the largest one in April. This was likely because older, larger females were able to spawn earlier in the season. Younger, smaller females might have been able to spawn later in the season, after gaining more resources to develop eggs.

Stage 3 ovaries were bright and distinctly colored. Yellowish eggs were seen as early as 14 January, but because they were not the dominant egg type, those ovaries were classified as Stage 2. However, other specimens collected in January and February had full of such eggs, so were classified as Stage 3. These would be expected to mature to Stage 4 and spawn in March or April, and we did find Stage 5 ovaries (spawned) beginning in those 2 months. However, most of the Stage 3 ovaries appeared in March and April.

The average TL of females with Stage 3 ovaries appeared to increase over the spawning season (Table 1). This seems to be in contrast with observations for Stage 2 females. However, we hypothesize that early spawners might have been able to spawn again within the season. If this was true, females that are larger in the latter part of the spawning season may be individuals delivering a second clutch (a group of eggs laid at the same time for fertilization). This hypothesis is supported by the fact that many of the individuals classified with Stage 3 ovaries in March and April were of greater TL, often over 100 mm. Two Stage 3 individuals collected at a late date of 4 June were 115 and 120 mm TL.

Further, Stage 3 ovaries very often contained white eggs consistent with Stage 2 ovaries, and Núñez and Duponchelle (2008) noted that Stage 3 and 4 ovaries containing a mixture of developmental stages of ova indicate multiple spawners. With the exception of the 2 individuals collected on 4 June, Stage 3 ovaries were not seen after 23 April.

Stage 4 ovaries were dominated by fully yolked ova, considered to be ripe and ready to be fertilized. We found such ovaries as early as 17 January and through 24 May. This stage was most common in April and May, indicating the peak of spawning for this species (Fig. 2). January and February specimens with this stage of ovary tended to be small, but the average size was consistent from March to May (Table 1). The range in TL of specimens with Stage 4 ovaries was greater in April and May. This is consistent again with the notion of multiple spawners, as some larger specimens probably laid a second clutch of eggs while some smaller individuals might have just come into full breeding condition. Most of these ovaries also contained ova in both of the earlier stages of development, suggested by Núñez and Duponchelle (2008) to represent multiple spawners. Such ovaries with eggs in multiple stages of development will release ripe eggs for oviposition while immature eggs are held within the ovary until they are matured, at which point they are released in a second spawn.

Table 1. Sizes of female Campostoma spadiceum (mm, TL) at each ovarian stage per month of the spawning season in southern Arkansas.

<table>
<thead>
<tr>
<th></th>
<th>Jan</th>
<th>Feb</th>
<th>Mar</th>
<th>Apr</th>
<th>May</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stage 2</strong></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>9</td>
<td>9</td>
<td>3</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>71.6 ± 3.4</td>
<td>65.9 ± 6.2</td>
<td>55.0 ± 3.6</td>
<td>55.6 ± 2.0</td>
<td></td>
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<tr>
<td>Range</td>
<td>60-87</td>
<td>55-113</td>
<td>50-62</td>
<td>49-61</td>
<td></td>
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<tr>
<td><strong>Stage 3</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>3</td>
<td>12</td>
<td>19</td>
<td>16</td>
<td>0</td>
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<tr>
<td>Mean ± SE</td>
<td>71.0 ± 8.0</td>
<td>74.8 ± 5.4</td>
<td>78.7 ± 5.0</td>
<td>80.5 ± 4.6</td>
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<tr>
<td>Range</td>
<td>63-87</td>
<td>49-106</td>
<td>55-125</td>
<td>55-128</td>
<td></td>
</tr>
<tr>
<td><strong>Stage 4</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>2</td>
<td>5</td>
<td>7</td>
<td>21</td>
<td>13</td>
</tr>
<tr>
<td>Mean ± SE</td>
<td>59.5 ± 0.5</td>
<td>73.8 ± 4.3</td>
<td>73.0 ± 4.2</td>
<td>73.0 ± 2.8</td>
<td>70.0 ± 3.8</td>
</tr>
<tr>
<td>Range</td>
<td>59-60</td>
<td>64-89</td>
<td>60-93</td>
<td>55-98</td>
<td>59-111</td>
</tr>
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</table>
hatched. Individuals too small to become sexually mature last season could mature earlier in the next season, and those hatched later in a previous year also matured later. This effect would extend the length of the spawning season for this stoneroller.

Stage 5 ovaries, representing the post-spawning condition, appeared by 2 March (Fig. 2). Stage 5 ovaries in which the body cavity was expanded but intestines had not refilled the vacated space were found on 2 March, 2 April, 4 May, 15 June, and 30 July. These observations indicate spawning events from March through July, but most specimens had completed spawning by late May.

Nuptial tubercles appearing on the head and body of males correlates with sexual development. We observed that the first bilateral pair of tubercles developed just in front of the eyes, followed by other pairs behind the eyes and continuing on the head. As the head became fully tuberculated, the structures began to develop centered along the anterior dorsal scales. A specimen collected on 21 November (65 mm TL) was just developing the first pair of tubercles. Larger specimens (80-135 mm TL) had full head tuberculation by mid-January, while smaller individuals (54 mm TL) had developed only 1-2 pairs at the same time. From February through May, males > 90 mm TL typically were fully tuberculated on the head and dorsal scales, whereas the smaller individuals tended to lack tubercles on dorsal scales and some of those typical for the head. Tubercles were found forming on the heads of smaller males (52-60 mm TL) on 17 January, 21 February, 8 March, 2 April, 10 April, 20 April, 24 May, and 5 June. Similar to reproductive development seen in females, we suggest that the > 4 month difference in these small but sexually maturing individuals represents the time when they were hatched. Individuals produced earlier in a previous year could sexually mature earlier in their first reproductive season, and those hatched later also matured later in a following season.

We speculate that the small females with ripe eggs should be able to deliver them into a spawning nest created by the large, dominant males. However, it is unknown whether the smaller males that show characteristics of sexual maturity are able to spawn. It is unlikely that they could compete with larger males for spawning sites. It would be interesting for further research to determine whether smaller males act as “sneaker” males to fertilize eggs deposited into the nests of large males (Lennon and Phillip 1960; Taborsky 1994; Stoltz and Neff 2006).

Although the length of the spawning season for C. spadiceum may be extended by multiple spawns, variation in seasonal temperatures also may affect the length of the breeding season. In New York, C. anomalum begins nest-building or territorial behavior when water temperatures reach 13-16°C (Miller 1964), Robison and Buchanan (1988) commented that spawning in Arkansas begins in March and April when water temperatures exceed 14.6°C (58°F), and Etnier and Starnes (1993) noted that peak spawning in Tennessee occurs at 12-14°C. Waters warmed earlier in a given year result in earlier onset of reproduction (but do not shift or truncate the season), therefore yearly variation in the arrival of spring temperatures can extend the period of reproduction (South and Ensign 2013).

We examined average monthly air temperature for January, February, and March, and compared it with normal temperatures (available from 2007-2018; www.usclimatedata.com/climate/arkadelphia/arkansas/united-states/usar0016). Assuming a strong correlation between air and water temperatures, we attempted to infer whether yearly and monthly temperature variation might induce variations in the timing of reproductive development. Of the 12 years of data, 3 years had warmer than average beginnings (for all 3 months), 4 had cooler than average beginnings, and 5 were mixed. Because most of our specimens were collected in the 1990-2005 period, we could not match specimen data with the available climatological data. However, available climate data reveal that earlier development is likely during some years, and delayed in others. The reproductive season reported herein represents a composite of seasons beginning earlier or later due to yearly variations in seasonal temperatures.

Acknowledgments

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Literature Cited

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