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Life History Characteristics of the Turquoise Darter (Etheostoma inscriptum) in the Upper Piedmont of South Carolina

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LIFE HISTORY CHARACTERISTICS OF THE TURQUOISE DARTER (*ETHEOSTOMA INSCRIPTUM*) IN THE UPPER PIEDMONT OF SOUTH CAROLINA

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ABSTRACT

The intention of this study was to estimate population density, time of spawning, and fecundity of the turquoise darter (*Etheostoma inscriptum*) in Six Mile creek after a translocation effort initiated in 2003. A population density study was conducted on 18 riffles within the lower 5000 ft of the creek starting approximately 1000 ft above its confluence with Lake Issaqueena from January to April 2008. Population estimates per m^2 were determined using the Leslie Depletion method. Random point collection was performed From October 14 to November 13, 2008 and 301 specimens were measured and released to determine length frequency of the population. Further length frequency data was collected from 314 specimens from March 3-13 2009, and sex was determined for additional sex ratio data. Sex ratio, fecundity, gonosomatic indices (GSI) and time of spawning were examined from January through late June 2009 by capturing and processing approximately thirty specimens per sample in roughly three-week intervals. Leslie depletion estimate of riffle density was 0.37 ± 0.16 darters/m^2, suggesting an established population. *E. inscriptum* were present in all riffles sampled. Length frequency data from spring 2009 collections resulted in three age classes for females and four for males. In the spring, sex ratio favored females 1.8:1. Male weight and length were significantly larger than females. Peak GSI for females occurred in April followed by a decline in May. GSI continued to decline through June, when spawning appeared to end. Fecundity analysis showed a continuum of ova maturation during the spawning period, suggesting multiple spawns. Annual fecundity estimates ranged from 122 to 235 ova/yr.

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LIFE HISTORY CHARACTERISTICS OF THE TURQUOISE DARTER (*ETHEOSTOMA INSCRIPTUM*) IN THE UPPER PIEDMONT OF SOUTH CAROLINA

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ABSTRACT

The intention of this study was to estimate population density, time of spawning, and fecundity of the turquoise darter (*Etheostoma inscriptum*) in Six Mile creek of the Clemson SC experimental forest. Population density estimates were conducted on 18 riffles within the lower 1,524 m of the creek starting 304.8 m above its confluence with Lake Issaqueena from January to April 2008. Density was determined using the Leslie Depletion method, and darters/m^2 was calculated. Random point collection was performed From October 14 to November 13, 2008, and 301 specimens were measured and released to determine length frequency of the population. Further length frequency data was collected on 314 specimens from March 3 to 13 2009, and sex was determined. Sex ratio, fecundity, gonosomatic indices (GSI), and time of spawning were examined from January through late June 2009 by capturing and processing approximately 30 specimens per sample in roughly three-week intervals. Leslie depletion estimate of riffle density was 0.37 ± 0.16 darters/m^2. *E. inscriptum* were present in all riffles sampled. Length frequency data from spring 2009 resulted in three age classes for females and four for males. In the spring, sex ratio favored females 1.8:1. Male weight and length were significantly larger than females. Peak GSI for males was the April 2 sample (1.1%), declining considerably by June 19 (0.4%). Peak GSI for females occurred in April followed by a decline in May. GSI continued to decline through June, when spawning appeared to end. Fecundity analysis showed a continuum of ova maturation during the spawning period, suggesting multiple spawns. Annual fecundity estimates ranged from 122 to 235 ova/yr.

INTRODUCTION

Numbers of species and populations of fish are declining due to the considerable impact of numerous stressors largely originating from anthropogenic origins. The Southeastern United States is decidedly one of the Earth's most diverse places of fish fauna representing 47% of the total North American species and 62% of the species in the United States (Warren et al., 1997). Of these species, 19% are listed as species of concern, threatened, or endangered (Fleischman, 1996). Habitat alteration instigates much of the loss due to the specific environments that some sensitive species need to exist. The family Percidae is represented by 149 species, of which 46 darter species are imperiled to some degree; many of which exist only within a narrow set of parameters in some of the most vulnerable ecosystems (Fleischman, 1996). Fragmentation of aquatic habitat and subsequent extirpation of isolated populations within a particular species range further jeopardizes population stability and may lead to extirpation or extinction (Warren et al., 1997). Alterations in stream geomorphology, introduction of chemical contaminants through non-point source runoff, and sedimentation of stream beds from poor land management practices all contribute to disruption of natural processes and threaten biodiversity throughout the ecosystem (Drennen, 2001; Tipton et al., 2004).

Lack of knowledge concerning the ecology of the affected species exacerbates the problem and may result in rapid decline or loss of the species before the realization of its endangerment. To forego these outcomes, a study of population dynamics is invaluable in the understanding of the species and in implementing appropriate management practices (Burkhead and Walsh, 2000). Life history studies are an important facet in a better understanding of the ecological components and habitat characteristics required for population stability (Bibb et al., 2000). Analysis of population density and reproductive components such as sex ratio, fecundity, and time and duration of spawning give basic information that can be applied towards conservation and management practices of the species (Knight and Ross, 1992).

The turquoise darter (*Etheostoma inscriptum*) naturally occurs in the Savannah River basin in North Carolina, South Carolina, and Georgia, and in the upper Ogeechee and Altamaha river basins of Georgia. Its status is considered stable in most of the area it occupies, excluding North Carolina where it is considered critically imperiled due to a limited range for the state (Hayes and Bettinger, 2005). Despite local abundance, *E. inscriptum* faces a possible decline due to urbanization impeding upon its narrow range (Baker, 2002). Anthropogenic disturbances in the early 1900s have functioned to extirpate the darter from Six Mile creek in what is now the Clemson University Experimental Forest (CUEF) (Kubach, 2003). The watershed surrounding Six Mile creek was stripped by years of extensive cotton farming prior to the 1940s and utilized as a bombing range during World War II (Sorrells, 1984). Construction of the Issaqueena Dam in the late 1930s (Dunn and Holiday, 1977) has served as a physical barrier for natural reestablishment. Adjacent streams within the region, such as Twelve Mile creek to the east, are populated with the species.

The prospect of a previously extirpated, sensitive species reestablishing a population within a rehabilitated aquatic community may serve as an example in the translocation of similar species in an attempt to maintain the highest level of biodiversity (Kubach and Foltz, 2003). Successful translocations have been conducted before on species such as the fringed darter (*E. crossopterum*) in Illinois, to establish feasibility and to suggest an alternative for the management of imperiled species such as barrens darter(*E. forbesi*) and duskytail darter (*E. percnurum*) (Poly, 2002). The Six Mile creek study may also facilitate conservation efforts in management for similarly imperiled species. Kubach's (2003) translocation efforts from February to March 2003 in Six Mile creek consisted of 80 sub-age 1 individuals. This founder population was monitored throughout the following summer months to ascertain survivorship. The objective of the current study was to determine the heretofore unknown population dynamics of this species, and the state of *E. inscriptum* in Six Mile creek since the translocation effort in 2003. This study sought to determine density, length frequency for age classes, sex ratio, fecundity, and gonosomatic indices (GSI) of the Six Mile creek population.

STUDY SITE

The location of the translocated population consisted of a 1,524 m, fourth-order stream section of Six Mile creek starting at a point of origin 274.3 m above the confluence with Lake Issaqueena in the CUEF. The study site occurs northwest of the Twelve Mile creek drainage, which supplied a portion of the specimens used for translocation (Kubach, 2003). The creek flows in a southwest trajectory through the CUEF until its convergence with the 43-ha Lake Issaqueena. It functions as the drainage basin for 36 km^2 of the Six Mile creek watershed in the Seneca hydrological unit within western Pickens County (Kubach, 2003). The experimental forest is a 12,005 ha plot of forested land acquired by Clemson University during the Land Use Deal (Straka et al., 2005). Previously these lands were deforested for use as crop lands and were badly eroded. The forest is now in a state of early succession consisting primarily of short leaf and loblolly pines, with an early stage emergence of hardwoods (Kubach, 2003). The river is flanked by a considerably shaded riparian zone consisting of mountain laurels and pines throughout the length of the study area (Kubach, 2003). The forest is utilized seasonally by the public for its proximity to Lake Issaqueena and its hiking trails, shelters, as well as recreational roads located throughout for access (Kubach, 2003).

The creek was demarcated into 30.5 m intervals posted with 2.5 cm PVC markers and divided into five, 304.5 m sections with 7.6 cm PVC markers during the translocation by Kubach (2003). Additional stream measurements were conducted in undergraduate research from fall 2008 through spring 2009, extending markers to 2,133.6 m from the origin, and ultimately expanding the sampling range. The first section was designated as the span from the point of origin located 274.3 m above Lake Issaqueena to the bridge crossing of Issaqueena Lake road. Section two encompassed the distance from the bridge to the first recreational road crossing. Subsequent sections spanned 304.8 m each.

METHODS

Population Density

Population density estimates of *E. inscriptum* at Six Mile creek were conducted from January 23 through March 24, 2008. Eighteen riffles were sampled in the initial 1524 m expanse of the creek. Riffles were designated as the sampling microhabitat in accordance with previous studies in habitat preferences for the species (Delong, 1991; Henry and Grossman, 2007). Capture procedures were approved by the Clemson University Institutional Animal Care and Use Committee (Animal Use Protocol ARC2007-082 teaching fisheries and ARC2007-086 darter restoration and assessment).

Block nets with 6 mm mesh were utilized to close the sampling area and collect any stunned fish missed by the netters**.** A Smith-Root Type VII Electrofisher (Vancouver, WA) was operated at approximately 60 Hz, 6 ms to maintain a current of 0.125 to 0.150 amps throughout the session (Cooke et al., 1998). Four to 5 assistants equipped with dip nets acted as collectors on either side and behind the individual utilizing the electroshocker in a sweeping method. Nets were checked approximately every 15 seconds to reduce harmful effects on specimens collected (Cooke et al., 1998). Bycatch was not recorded, as sampling for fish assemblage in Six Mile creek has been conducted by Foltz numerous times from 2002 through 2007 (Appendix). Typically the width of the creek indicated the need for one single or a divided left and right double pass. Multiple passes, typically 3 to 4, were performed as needed over the entire area until few or no fish were collected. Number of *E. inscriptum* collected from each pass was counted and the effort per unit time was recorded. Fish collected in each pass were contained in separate 11-L holding containers in approximately 4 L of water until fully recovered; and after completion of all successive passes, they were then released at the head of the riffle. Water temperature ($T^{\circ}C$) and dissolved oxygen concentration (DO in mg/L) were recorded for each sampling location using an YSI Model 85 handheld dissolved oxygen, conductivity, salinity, and temperature meter (Tampa, FL). Number of fish per square meter for each riffle was calculated.

Length Frequency

Length frequency data for fall were collected over a four week period from October through November 2008. Spring data were collected from March 3 to 13, 2009. Random point collection was performed in riffles throughout the 2,133.6 m expanse of the study area using a single upstream pass and either sweeps or spot electrofishing as microhabitat dictated. Fish were placed in a holding container until recuperated and measurements in total length (TL) to the nearest mm were recorded. In the spring 2009 collection, sex was also determined by the presence of external genital papilla in females. All fish were released upon completion of the sampling for the given area. Stream temperature and DO were also recorded.

Sex Ratio, Gonosomatic Indices and Fecundity

Fecundity estimates and additional data for sex ratio were collected from January through June 2009, with a capture of approximately 30 specimens at 3 to 4 week intervals for a total of eight sample dates. A follow-up sample was undertaken August 18, 2009 for detection of young-of-the-year and reproductive condition of adults. Specimens were collected by electrofishing, and fish were euthanized at collection using buffered 1000 mg/L MS-222 (tricaine methanesulfanate). Stream temperature and DO were recorded. Specimens were rinsed and individually frozen until examination.

Specimen TL was measured, and wet weight after blotting dry was recorded to 0.01 mg using a Mettler H51 Balance (Hightstown, NJ). Sex was determined by both the presence of external genital papilla and verified by gonad assessment upon dissection. Ovaries and testes were removed, blotted dry, and weighed. Gonosomatic indices (GSI, gonad mass as a percentage of body mass) for each sex were examined for the eight collection dates. Ova maturation and egg counts were performed on dissected ovaries with a Zeiss Stemi 2000-C dissection microscope (Toronto, ON). Fecundity was examined by dissection of ovaries for total egg count. Mature eggs started to accumulate by April, at which time both immature and mature totals were tabulated. Mature eggs were distinguished by homogenous size ($\geq$ 1.5 mm in diameter) and elevation of the vitelline membrane (Layman, 1993) (Figure 1).

Statistical Analysis

Density estimates for each riffle were determined using the Leslie depletion method (Ricker, 1975). All statistical analyses were performed using SAS software (Statistical Analysis Software Cary, NC) with an α=0.05 where applicable. Modal separations for division of age classes were analyzed using nonparametric kernel density estimation (Wand and Jones, 1995). Sex ratio was analyzed using a Chi-squared test for data obtained from both the length frequency and fecundity data sets for the largest sample size. Analysis of differences in mean length and weight (minus gonad wt.) of males versus females was performed using a general linear model (GLM) with Tukey's Studentized range distribution. The GSI estimates from January through June samples were analyzed statistically using a GLM and Tukey's. Linear regression was performed on length of females as compared to egg production from January 21 to April 23 (proposed pre-spawning period) to determine if there is a relationship between TL and fecundity. Total fecundity per month was analyzed by date using a GLM. Numbers of both immature and mature ova from collection date of peak GSI through final collection date were examined separately using a GLM for each.

RESULTS

Population Density

Total capture of *E. inscriptum* for the 18 riffles sampled was 409. Total estimated population size ($\hat{n}$) for the studied area was 449. Individual riffle population estimates are recorded in Table 1. The coefficients of determination (R^2) for the Leslie fit regression were 0.97 or higher in 89% of the riffles sampled. Total mean proportion of all fish caught per pass $\pm$ 2SE was 72% for pass 1, 18% for pass 2, 9% for pass 3, and 1% for pass 4 (Figure 2). Mean density ($\pm$ 2 SE) was 0.37 $\pm$ 0.16 darters/ m^2. Highest density occurred in smc-3-rf-6 with an estimated 1.33 darters/ m^2, and lowest density was smc-1-rf-2 with 0.01 darters/ m^2.

Length Frequency

Length frequency data for fall 2008 resulted in a bi-modal histogram (Figure 3). Spring 2009 data as divided by sex (males n=111, females n=204) demonstrated 3 age classes in females (0, 1, and 2) offset by 4 age classes in males (0, 1, 2, 3) (Figure 4). Age class 0 peaked at 39 to 41 mm TL for females and 45 to 47 mm for males. Age class 1 for females and males peaked at 48 to 50mm and 51 to 53 mm respectively. The age class 2 peaks for females and males were 54 to 56 mm and 57 to 59 mm, respectively. Male age class 3 peaked at 66 to 68 mm. The longest darter collected was 77 mm TL in spring 2009.

Sex Ratio and Related Studies

Chi-square test results on sex ratio (n=530) revealed a significant difference from a 1:1 ratio. This resulted in a 1.8:1 ratio of females to males. GLM tests on both length and weight from euthanized specimens collected from January through June 2009 (n=243) were significantly different and revealed higher mean values (± 2SE) for males

(TL=55.3 ± 0.9 mm, weight=1.9 ± 0.9 g) than females (TL=49.4 ± 0.5 mm, weight=1.2 ± 0.1 g). Length - weight relationships for both sexes are displayed in Figure 5. Mean (± 2SE) lengths of males and females from length frequency data collected March 2009 (n=315) were 57.1 ± 0.8 mm and 50.4 ± 0.5 mm, respectively.

Gonosomatic Indices and Fecundity

The GLM with Tukey's grouping of male GSI for the eight sample dates from January to June 2009 demonstrated a significant difference between April 2 (1.10%) and June 19 (0.40%). Female GSI demonstrated more instances of significant difference than males with March 12 (8.86%) differing from January 21 and February 17 (3.81% and 5.54% respectively); April 2 (11.39%) differing from March 12; May 13 (9.35%) differing from April 23 (12.83%); and June 1 and 19 (5.51% and 3.08%, respectively) each differing from the previous date (Figure 6).

Regression analysis on egg production per TL demonstrated a significant relationship (Figure 7). Tukey's grouping analysis for differences in total ova for each collection date resulted in no significant difference in January 21 through April 23 (376, 339, 351, and 279, respectively) with the exception of January 21 (376) and April 2 (267) (Table 2). May 13 (230) was significantly lower than January (376). June 1 (111) and June 19 (44) were each significantly different than January through May. Fecundity as number of mean mature ova from April through June showed significant differences between April 2 and 23 (34 and 52, respectively), and May 13 and June 1 (52 and 19, respectively) (Figure 8). Immature ova from the same time period revealed a significant difference for May 13 and June 1 (178 and 92, respectively) (Figure 9). Monthly means of mature ova as compared to mean immature ova is demonstrated in Figure 10.

DISCUSSION

Population Density

The estimate of total population of each riffle ($\hat{n}$) using the Leslie depletion method demonstrated mean values of 0.37 turquoise darters/m^2, suggesting an established population from the time of translocation in 2003. Discrepancies in density estimates due to fright-bias were considered nominal due to the benthic nature of *E. inscriptum* and the physical character of the riffles (Bain and Finn, 1991). The number of turquoise darters per riffle was highly variable, ranging from 0.01 darters/m^2 to 1.33 darters/m^2, and may be a reflection of habitat preferences (Henry and Grossman, 2007). Microhabitat structure has shown to substantially effect density estimates for darters. Past analysis concerning suitability variables of water depth, substrate, and velocity indicated all three factors in combination being indicators of preferred habitat for *E. inscriptum*, with water velocity being the limiting factor in most of the sampled areas (Delong, 1991). In addition, studies on microhabitat characteristics have observed an increase in darter density in relation to macroinvertebrate abundance (Rakocinski, 1988). Information collected on other darters inhabiting riffles has reported substantial variation between species (Page, 1983). The fantail darter (*E. flabellare*) population densities range from 0.17 to 0.42/m^2 in Pennsylvania to 5.59/m^2 in southwestern Ohio (Schwartz, 1965; Mundahl and Ingersoll, 1983). Gilt darter (*P. evides*) densities in Macon County, North Carolina of 0.31 darters/m^2 (Skyfield and Grossman, 2007) were similar to the 0.37 turquoise darters/m^2 estimated in this study.

Length Frequency

An age class 0 was observed in the fall, but significant overlap occurred in subsequent age classes making class separation impossible. This failure was likely a result of differential growth rates between sexes, as observed in the following spring

(Figure 4). Spring data revealed distinct modal separation of age classes for each sex. Females revealed 3 age classes, with males having 4 age classes. A small percentage of the population exceeded three years of age. Simmons et al. (2008) observed a similar longevity in male bluemask darters (*E. doration*), and male longevity has been documented in redline darters (*E. rufilineatum*) (Widlak and Neves, 1985). No age class 3 males were observed in the August 18, 2009 sample. In both fall 2008 and spring 2009 samples, age class 0 had fewer darters than age class 1, while the August 2009 sample demonstrated a much stronger age class 0. Kernel density estimation was utilized to reduce the subjectivity inherent in length frequency analysis as an indicator of age classes. Determination of age in darters by analysis of annuli on scales has not always proven reliable (Taber et al., 1986; Ryon, 1986) and thus was deemed beyond the scope of this study.

Evidence of 2009 young-of-the-year was first observed June 19 with the capture and release of five juveniles approximately 12 to 15 mm TL. This roughly demonstrates the growth rate when compared to incubation period and hatching length of other darter species. GSI results indicate spawning started middle to late April and continued into early June. Time of incubation for the ova varies among species: the orangefin darter (*E. bellum*) has exhibited an incubation period of 7 to 9 days at a constant temperature of 23°C in captive conditions (Fisher, 1990) and Layman (1993) reported 6 to 11 days at 22°C in the savannah darter (*E. fricksium*). Darter larvae exhibit a range of TL upon hatching; studies performed on the least darter (*E. microperca*) estimated a hatchling TL of 3.5 to 3.8 mm (Johnson and Hatch, 1991), while Fisher (1990) reported a protolarvae TL of 6.1 mm in *E. bellum*. Based on the lengths of other darter species at hatching, 12 to 15 mm specimens of *E. inscriptum* encountered in June 2009 could be approaching up to two months of age when observed. This corresponds with the 2003 collection of four young-of-year approximately 20 mm TL on July 17, and one 32 mm specimen July 24, from post-translocation sampling in Six Mile creek (Kubach, 2003). The August 2009 sample was over 50% young-of-the-year, averaging 34.8 mm TL. Length frequency data collected fall 2008 reveals age class 0 to average about 40.0 mm (Figure 3); this would coincide with the growth rate of the new 0 age group from August 2009. Several other darter species have demonstrated a rapid growth rate in the first year. Flynn and Hoyt (1979) observed that the teardrop darter (*E. barbouri*) attains 66 to 68 % of maximum length within the first year of growth and 89 to 92 % of maximum at the end of year two. The bayou darter (*E. rubrum*) reportedly reaches 60% maximum TL at the first year (Knight and Ross, 1992). Male turquoise darters in age class 0 from the spring 2009 length frequency peaked at 46 mm; this would project a growth of 60% maximum total length in the first year, and corresponds with findings for other darter species.

Sex Ratio and Related Studies

Sex ratio for *E. inscriptum* was strongly in favor of females with a 1.8:1 ratio. Darter species have demonstrated a great deal of variety in this trait, with bluemask darter (*E. doration*) having shown a similar ratio of 1.8:1 females to males in Central Tennessee (Simmons et al., 2008), saddled darters (*E. tetrazonum*) have been observed to approximate 1:1 ratio (Taber and Taber, 1983) and both redline (*E. rufilineatum*) and fantail (*E. flabellare*) darters have exhibited ratios favoring males as a result of differential survival (Baker, 1978; Widlak and Neves, 1985). Sex ratio being skewed substantially towards females coincides with darter species that exhibit male territoriality in spawning season (Layman, 1993).

Size dimorphism was also exhibited in *E. inscriptum* as males were significantly larger in both length and weight than females in the population. This trait has been documented among darter species that exhibit male territoriality (Page, 1983). Hansen et al. (2006) reported larger mean lengths for male barrens darters (*E. forbesi*) and competition for nest sites, common with *E. nigripinne* and *E. crossopterum* in the spottail darter (*E. squamiceps*) complex. Additionally, *E. inscriptum* exhibited marked sexual dichromatism during the spawning season, and returned to a monochromatic state post-spawning, as observed in August. Nuptial colorations often function to attract females and establish breeding territories in many species of fish (Kodric-Brown, 1998), and have been observed in numerous species of darters (Page, 1983). This trait, along with sex ratio and size dimorphism, supports the hypothesis of male territoriality in turquoise darters.

Gonosomatic Indices and Fecundity

Mean GSI values for males varied less than females over the gonad maturation and spawning period. Male GSI values peaked April 2, just prior to the female's peak of April 23. The GSI remained elevated, although diminishing over time, until dropping considerably by mid-June to a level less than half of that recorded in January. Female GSI values demonstrated a constant increase from January until the peak of April 23, after which each collection date was significantly less than the previous. Total female GSI for June 19 was less than January, as with the males. Temperature has been indicated as a factor in fish spawning; Weddle and Burr (1991) found that snubnose darters (*E. rafinesquei)* produced the largest clutches as stream temperatures approached 20°C, and documented cessation of spawning as temperatures regularly exceeded 20°C over successive days. In Six Mile creek, April 23 had the highest temperature (18.2°C) to date for spring, coinciding with peak GSI and the estimated spawning initiation (Table 2). Water temperatures were relatively unchanged at May 13 (18.3°C), and clutch size (as number of mature ova) was still high. June 2 stream temperature was 19.5 $^{\circ}$C and a decrease in ova numbers was observed. By June 19 temperatures had reached 22.3 $^{\circ}$C and spawning likely had reached completion.

Mature ovum were tabulated in examination of fecundity in addition to cumulative egg count; initially due to suspicion that the species may undergo multiple spawns as observed in other darter species (Ryon, 1986). This reproductive strategy can make estimates of total fecundity difficult to ascertain without knowledge of clutch size (Khudamrongsawat et al., 2005). Fecundity was highly variable amongst specimens, with similar length fish having substantially different total egg counts. However, number of ova produced by females showed a significant relationship to TL, suggesting production of ova increases in direct proportion to size (Figure 7). Minimum TL of dissected specimens with ova was recorded as 32 mm on January 21, with one 39 mm specimen developing mature ova recorded April 2. All sampled *E. inscriptum* in age class 0 appeared capable of spawning prior to reaching one year in age. January and February samples contained no mature ova. Females in the January 21 sample did possess alimentary adipose tissue absent in the February sample, and also had a notable lack of gut contents within the stomach and intestine as compared to males. The March 12 sample revealed maturation in a small percentage of ova, and subsequent collection dates tabulated both immature and mature oocytes. Mature ova counts peaked in accordance with the GSI date of April 23 (Figure 8) and remained elevated while GSI dropped. Mean immature ova counts also diminished from April 23 to May 13, suggesting recruitment into mature ova (Figure 9). June 1 values corresponded for GSI, mature ova counts, and immature ova counts and were significantly less for each (Table 2). As of the June 19 sample, eggs in a few ovaries were still discernable, while the majority of remaining mature and atretic oocytes were undergoing reabsorption with a breakdown of the cellular membrane, thus ceasing a reasonable summation of remaining eggs due to structural integrity (Figure 11). Of the sampled specimens, 12 of

16 ovaries were vacant or reabsorbing eggs, and a few specimens had begun to accumulate alimentary adipose tissue. Analyzing the differences in mean numbers of total ova in each sample from April 23 through June 19, fecundity as the number of eggs spawned could be estimated as a mean of 235 ova/yr. This method would suggest around 86% of eggs produced were spawned. However the length of time mature ova were present, while quantities of immature ova were diminishing along with the GSI, lends evidence to support the theory that turquoise darters are multiple spawners. Fecundity as total number of mature ova (or clutches) could be estimated from the sampling date means of April 23, May 13, and June 2 (51.4, 51.6, and 19.1 mean mature ova, respectively) and evaluated as three potential clutch sizes for a mean total fecundity of 122 ova/yr. This estimate is about half the total oocyte count, which would result in equally misrepresented totals. Bagenal (1978) defines fecundity as "the number of ripening eggs in the female prior to the next spawning period". To arrive at a more definite estimate of fecundity, spawning frequency may need to be observed. Weddle and Burr (1991) observed multiple spawns of snubnose darters (*E. rafinesquei*) using in-stream confinement methods, and determined estimates of spawned ova were similar to that of counts of mature ova from wild-caught specimens. However, throughout the length of the study, they also observed recruitment of immature oocytes into maturation, as well as the spawning of one clutch over several days as dictated by stream temperature. Gale and Deutsch (1985) reported similar recruitment of immature oocytes in tessellated darters (*E. olmstedi*), with 2 to 8 clutches deposited in 5 to 16 day intervals, and a similar conclusion that either total or mature egg counts may misrepresent annual fecundity without an observation of actual clutch size. In this study, immature ova of intermediate size after April 23 samples were observed but not separately quantified, as a continuum of sizes existed. Without observation of spawning and clutch sizes, the best estimate of the turquoise darter annual fecundity ranges from

122 to 235 eggs/yr. On a final note, August 2009 collections demonstrated latency in both testis and ovaries, with both sexes accumulating large depositions of alimentary fat. Sex was indistinguishable for young-of-the-year spawned in April, while examination revealed genital papilla were still apparent in females age class 1 and older.

Future Considerations

While population densities were established in riffles for the initial 1,524 m section of the study, the areas from 1,524 to 2,133.6 m produced a large amount of darters in each single electrofishing sweep of the riffle. Comparison of abundance upstream and a study of additional microhabitat variables might be beneficial in determining other indicators of habitat suitability or preference. At first appearance, the upper length of the study area was largely flanked by considerably more mountain laurel (*Kalmia latifolia*) than the lower reaches, which might provide additional cover. In addition, invertebrate abundance as a component of habitat may reveal a relation to substrate or water velocity and preferred habitat that was previously undetected, as well as gut analysis to determine diet for the species.

Currently, many aspects of spawning activity remain unknown. Observations in-stream and from captive specimens would offer insight into clutch size and frequency of spawning, resulting in a better estimate of total fecundity. In addition, weekly or daily monitoring of temperature for both in-situ and stream conditions may reveal the relationship between temperature and spawning, and provide necessary information for projections as to when spawning occurs. Studies on spawning behavior and selection of nest sites, including characteristics of substrate and method of egg application, would assist in defining key habitat requirements for the species. Evaluating the length of time for incubation in developing ova might uncover a relation with optimum temperature and embryo survival and ultimately time of spawning, as seen in several species

(Weddle and Burr, 1991). Larval traits, such as length upon hatching, growth rate, dispersal, and movements within the stream have yet to be determined. Knowledge gained from this project offers a glimpse into life history traits that were previously unknown concerning the turquoise darter, and brings into focus new questions about the reproduction and ecology of the species. Further discoveries will reveal important information that may be applied towards management of the species, and provide more insight into other precariously situated species and their inherent requirements for survival.

Table 1. Estimated number of *E. inscriptum* per m^2 for each riffle.

Site*	Fish/m^2	Total capture	Estimated population ($\hat{n}$)	R^2 of Leslie fit
smc-1-rf-1	0.03	6	6	0.99
smc-1-rf-2	0.01	1	1	1.00
smc-1-rf-3	0.08	12	12	0.99
smc-2-rf-2	0.02	2	2	1.00
smc-2-rf-3	0.43	22	26	0.99
smc-2-rf-4	0.16	16	19	0.97
smc-2-rf-5	0.40	34	42	0.99
smc-2-rf-6	0.83	37	41	0.99
smc-3-rf-1	0.62	61	61	0.99
smc-3-rf-3	0.35	26	36	0.99
smc-3-rf-4	0.61	28	30	0.99
smc-3-rf-5	0.29	23	26	0.93
smc-3-rf-6	1.33	37	40	0.97
smc-3-rf-7	0.27	31	31	0.99
smc-4-rf-1	0.21	15	16	0.99
smc-4-rf-3	0.38	26	27	0.99
smc-4-rf-4	0.48	20	20	0.99
smc-5-rf-1	0.14	12	13	0.99

*sites are designated as Six Mile creek (smc), section number, and riffle number per section.

Table 2. Records of temperature, GSI, mean total ova with Tukey's grouping and mean mature ova for female *E. inscriptum*, 2009.

Date (2009)	Temp (°C)	GSI (%)	Total ova*	Mature ova	*n*	$\overline{x}$ TL (mm)
1/21	4.1	3.80	376 A	-	12	50
2/17	7.1	5.54	339 AB	-	23	47
3/12	14.7	8.86	351 AB	-	17	50
4/2	15.0	11.40	267 B	34	20	49
4/23	18.2	12.83	279 AB	51	23	49
5/13	18.3	9.35	230 B	52	23	49
6/1	19.5	5.52	111 C	19	20	47
6/19	22.3	3.08	44 C	6	16	52

* Means sharing the same letter are not significantly different.

Figure Captions.

Figure 1. Mature eggs were distinguished by homogenous size and elevation of the vitelline membrane

Figure 2. Proportion of catch per pass with 2 SE for *E. inscriptum* spring 2008

Figure 3. Length frequency averages in 3 mm increments for *E. inscriptum* for March 2009 (n=307)

Figure 4. Age classes and Length-frequency in 3 mm increments of male vs female *E. inscriptum* for March 2009 (n=315).

Figure 5. Length-weight relationships for Female (A) and Male (B) *E. inscriptum*

Figure 6. Mean GSI with 2 SE for Female (A) and Male (B) *E. inscriptum* per collection date

Figure 7. Number of ova per length for *E. inscriptum*

Figure 8. Mean mature ova with 2 SE for *E. inscriptum* April thru June 2009

Figure 9. Mean immature ova with 2 SE for *E. inscriptum* April thru June 2009

Figure 10. Proportion of mature to immature ova for *E. inscriptum* April thru June 2009

Figure 11. Ova reabsorption June 2009.

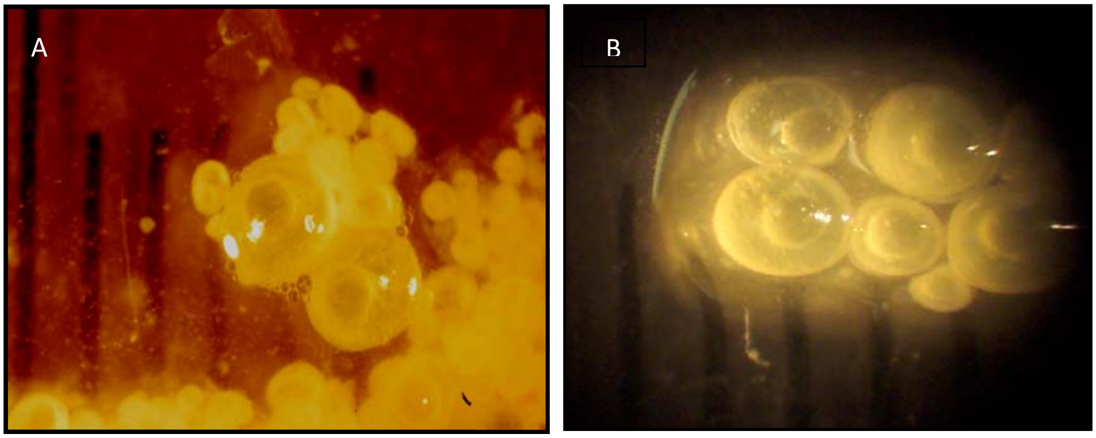

Figure 1. Mature ova were distinguished by homogenous size and elevation of the vitelline membrane. A. demonstrates two mature and numerous immature ova; B. demonstrates stages of maturing ova.

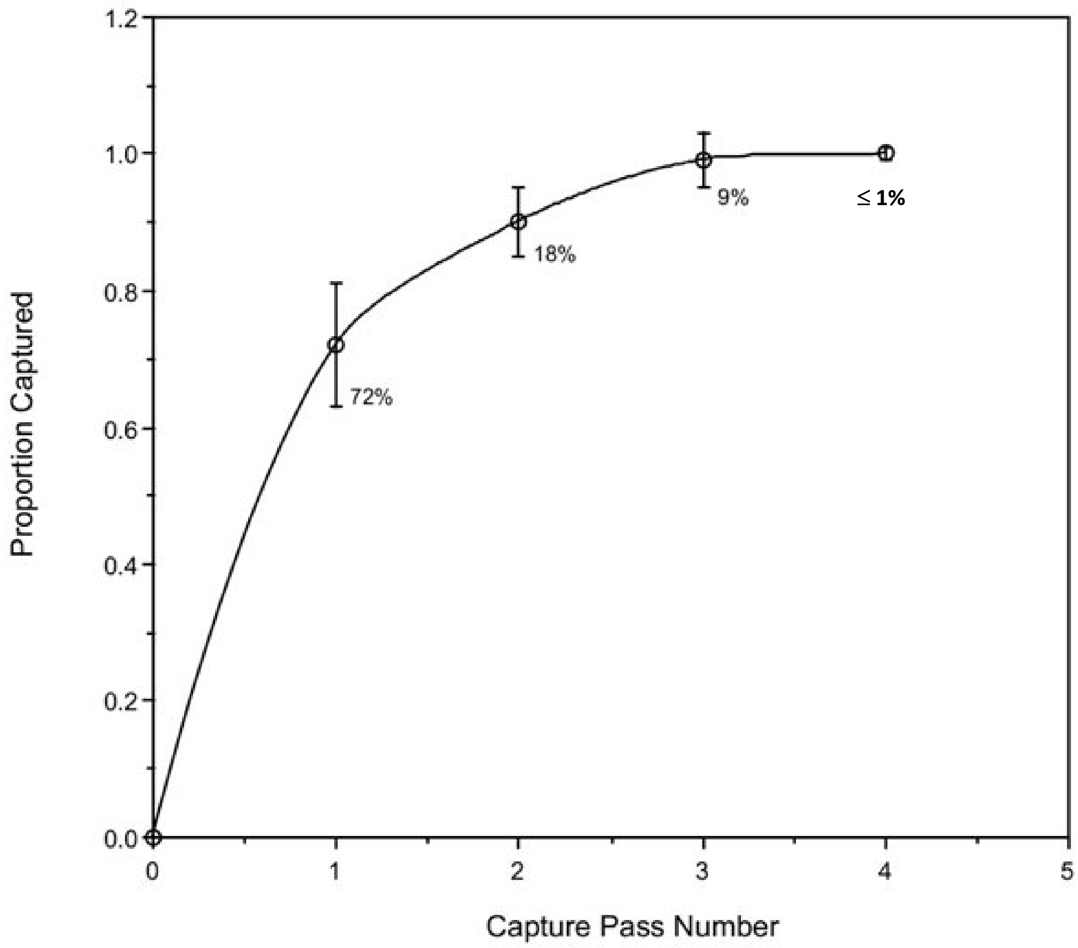

Figure 2. Mean proportion (±2SE) of catch per pass for *E. inscriptum* spring 2008.

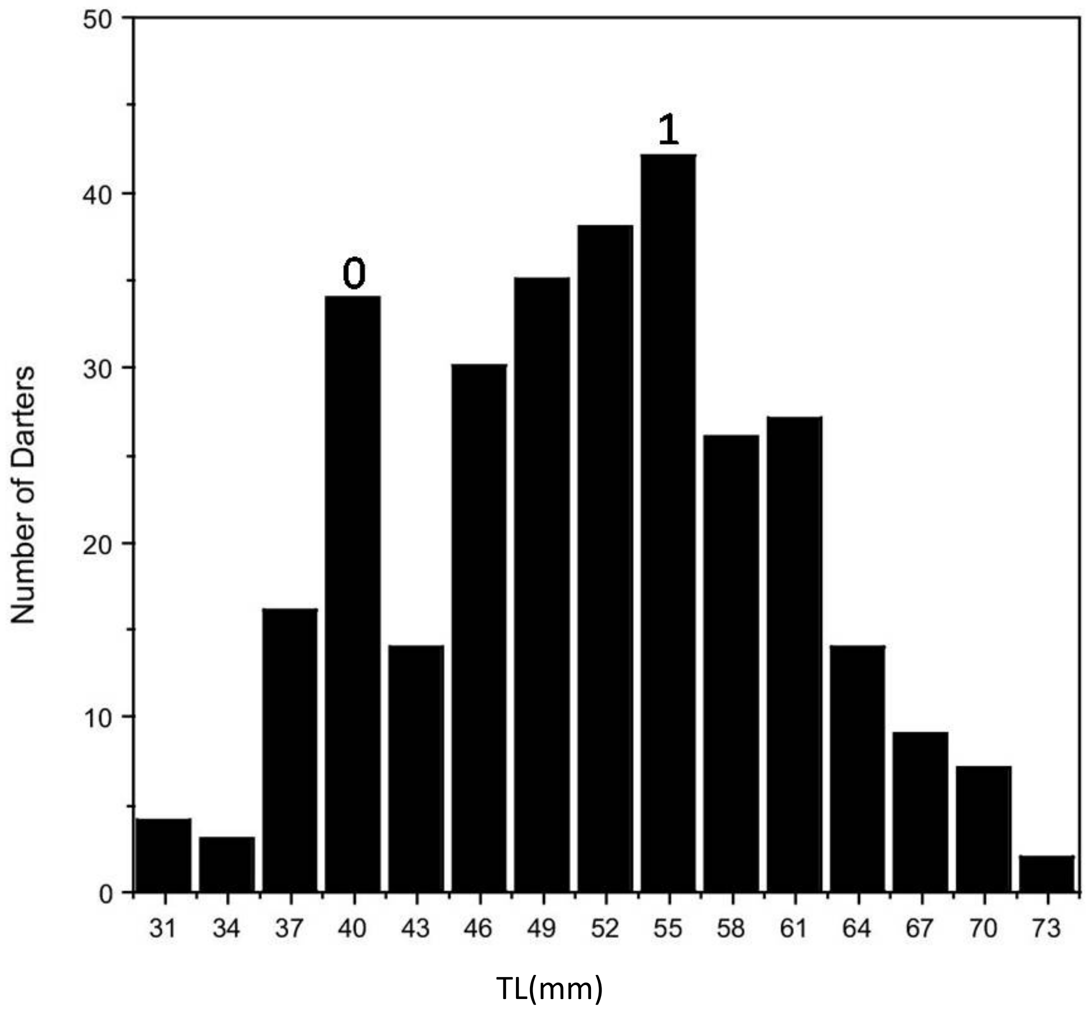

Figure 3. Age classes and length frequency in 3 mm TL class intervals for *E. inscriptum* (n=307) for fall 2008 collection.

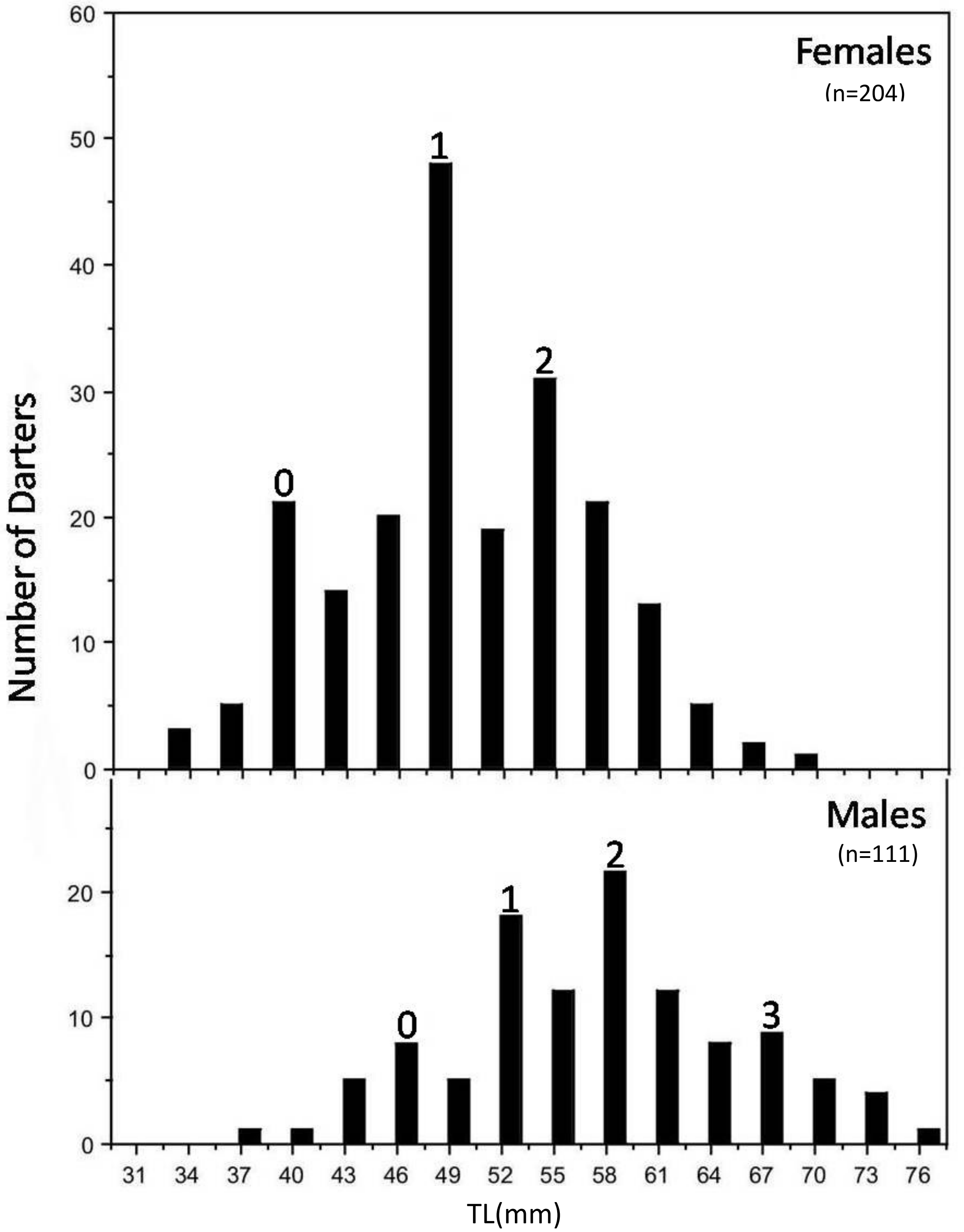

Figure 4. Age classes and length frequency in 3 mm TL class intervals of male vs female *E. inscriptum* for March 2009 collection.

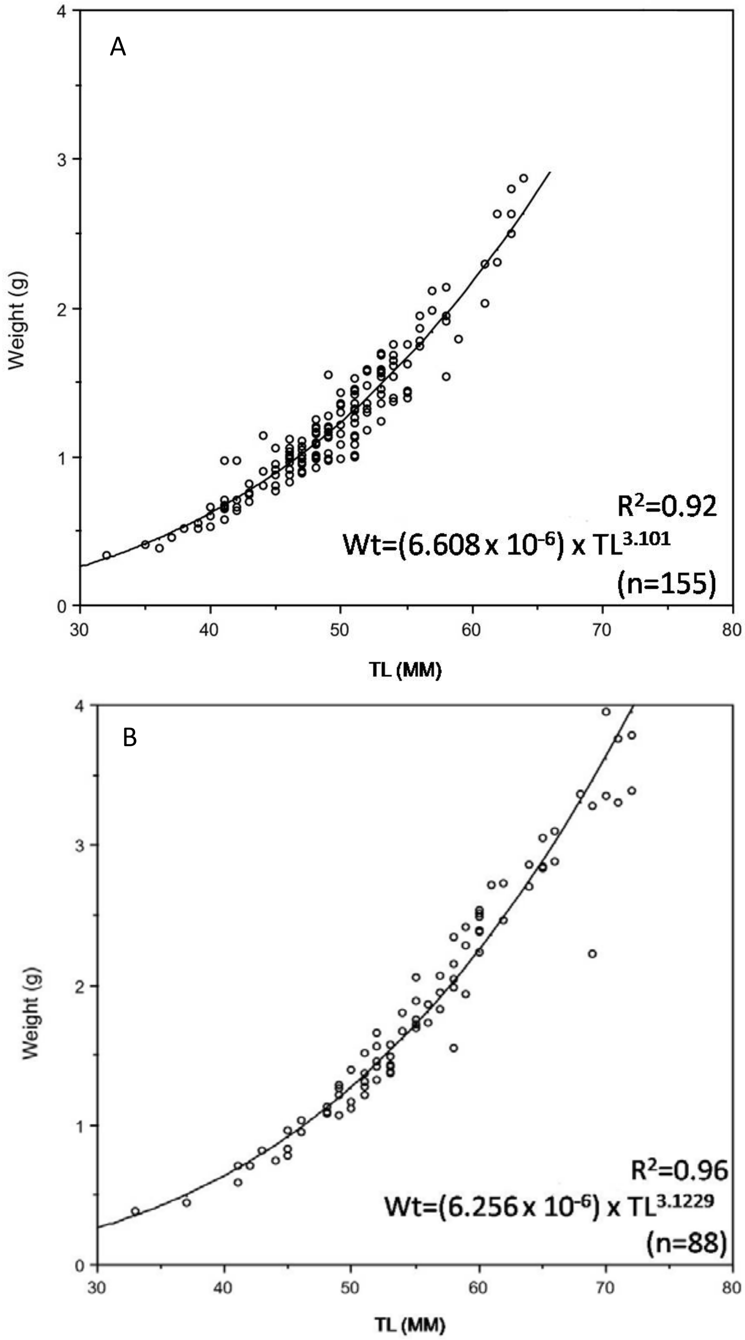

Figure 5. Length-wt relationships for female (A) and male (B) *E. inscriptum.*

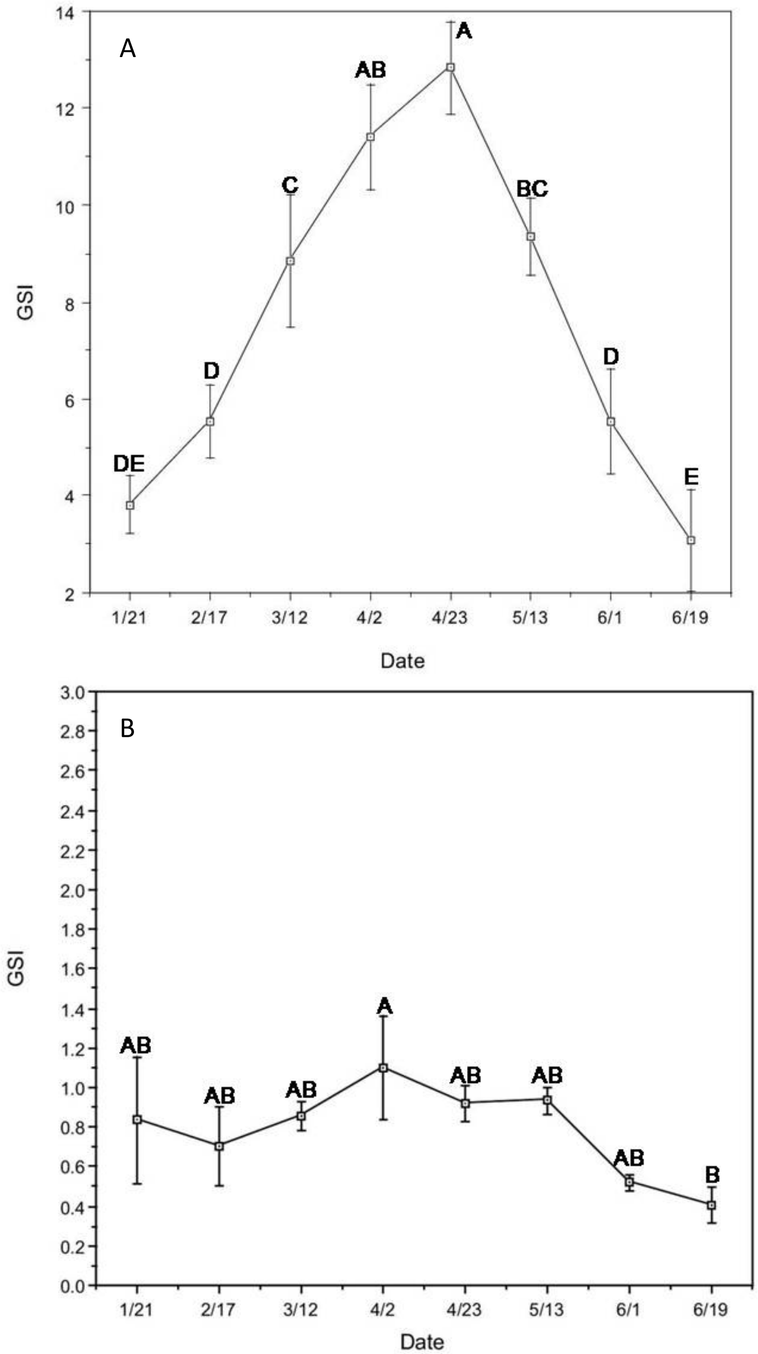

Figure 6. Mean (±2SE) GSI with for female (A) and male (B) *E. inscriptum* by 2009 collection date. Means sharing the same letter are not significantly different.

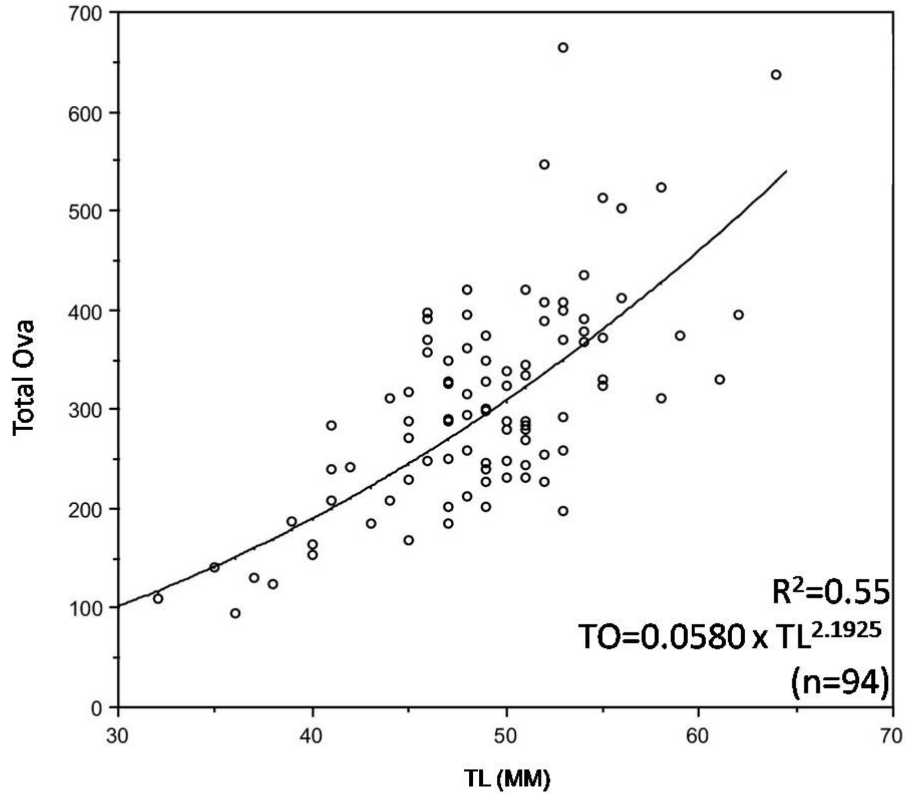

Figure 7. Number of total ova per TL for *E. inscriptum.*

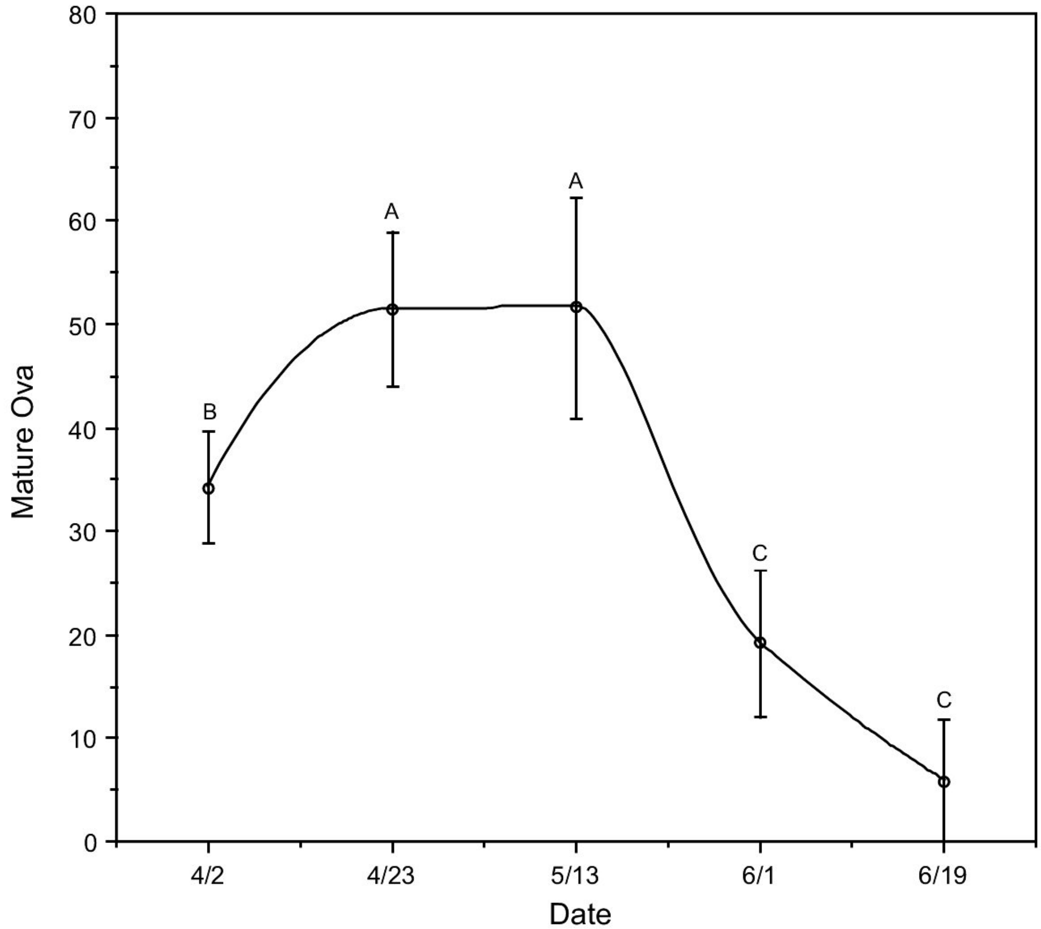

Figure 8. Mean (±2 SE) mature ova with Tukey's grouping for *E. inscriptum* April through June 2009. Means sharing the same letter are not significantly different.

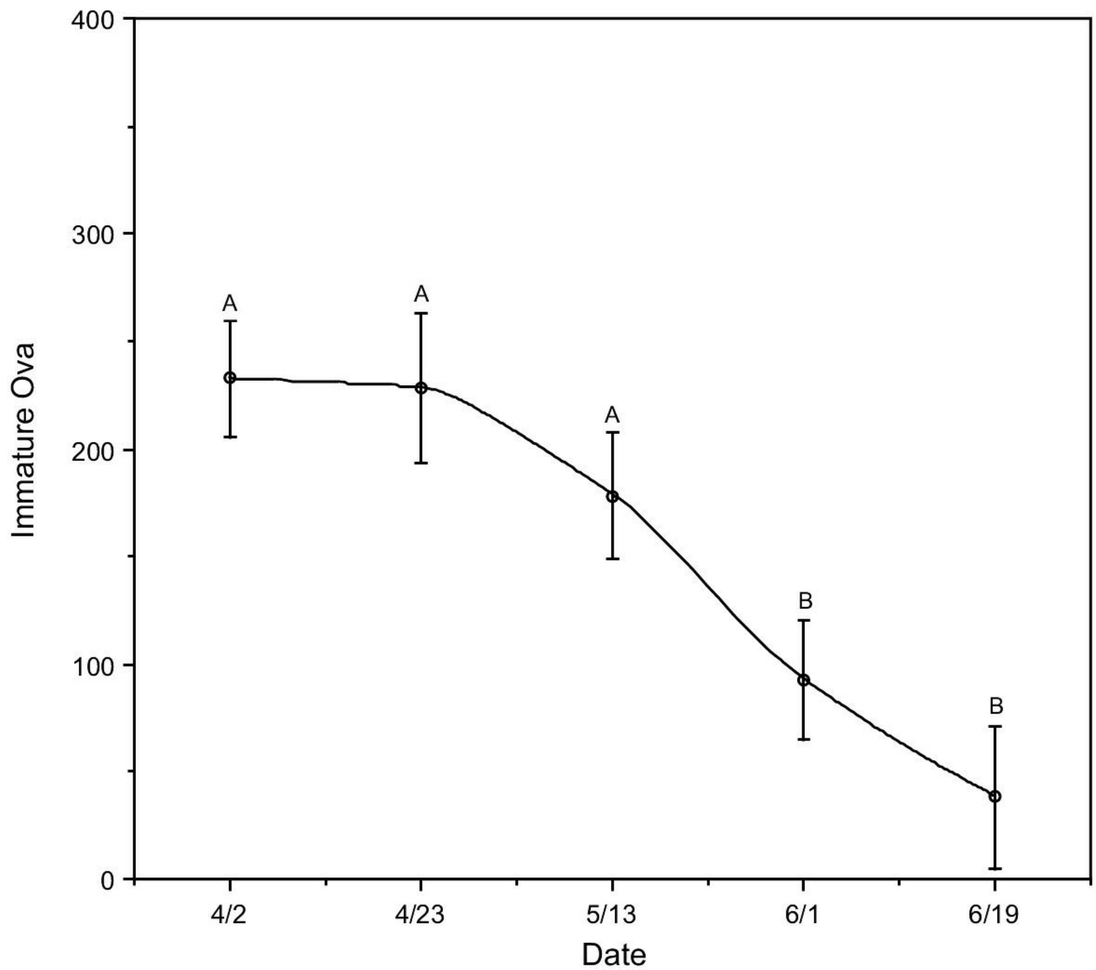

Figure 9. Mean (±2 SE) immature ova with Tukey's grouping for *E. inscriptum* 2009. Means sharing the same letter are not significantly different.

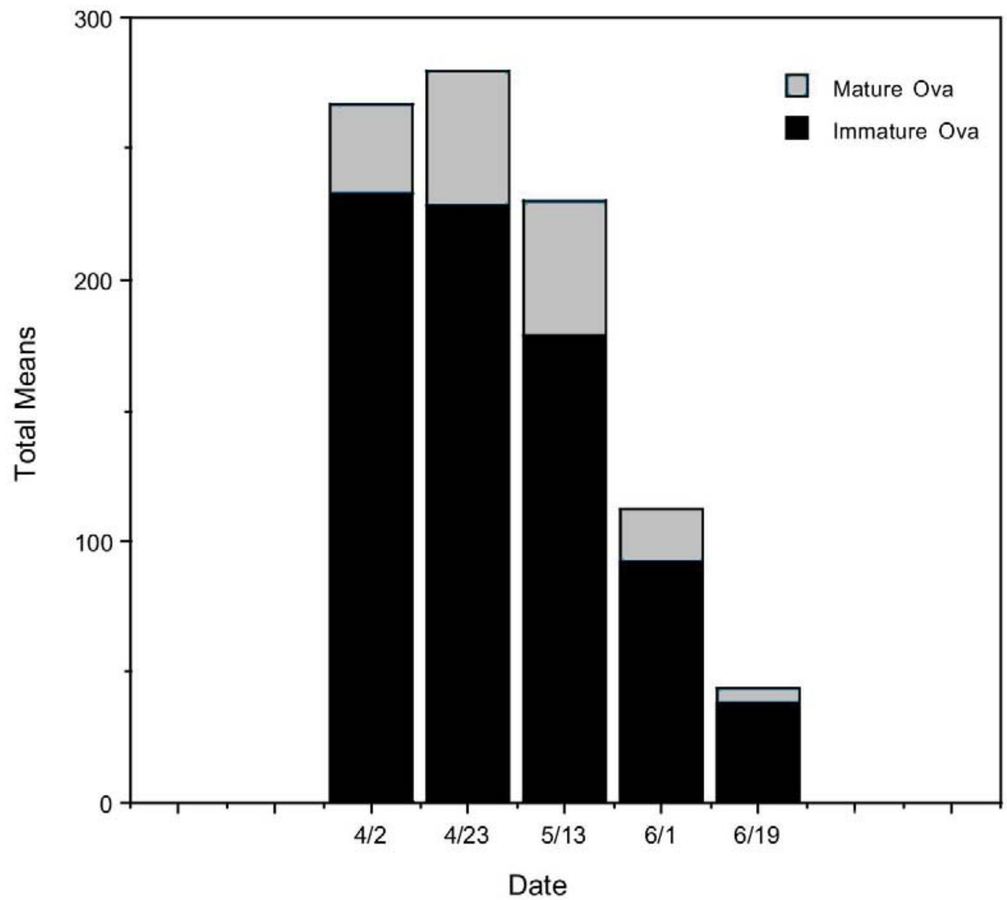

Figure 10. Number of mature to immature ova for *E. inscriptum* April through June 2009.

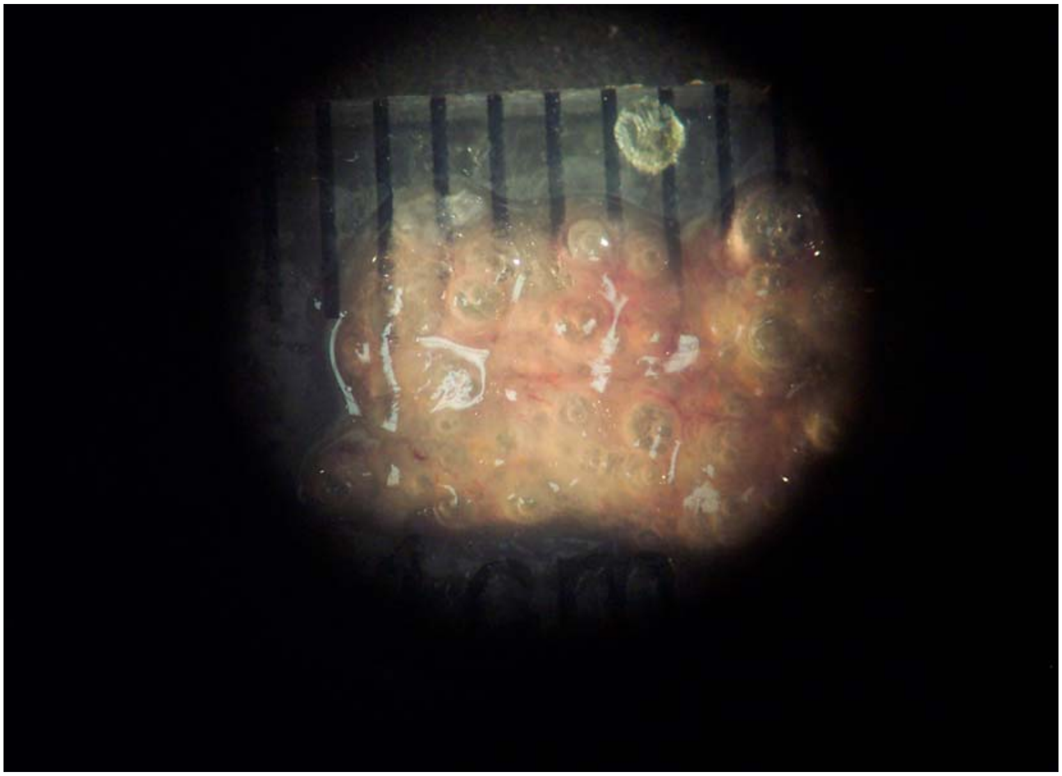

Figure 11. Ova reabsorption June 2009.

APPENDIX

Documented species present in Six Mile creek from 2002 to 2007 (Foltz, 2009).

Common Name	Scientific Name
Chain Pickerel	*Esox niger*
Yellowfin Shiner	*Notropis lutipinnis*
Bluehead Chub	*Nocomis leptocephalus*
Creek Chub	*Semotilus atromaculatus*
Rosyface Chub	*Hybopsis rubrifrons*
Whitefin Shiner	*Cyprinella nivea*
Northern Hogsucker	*Hypentelium nigricans*
Striped Jumprock	*Scartomyzon rupiscartes*
Margined Madtom	*Noturus insignis*
Speckled Madtom	*Noturus leptacanthus*
Yellow Bullhead	*Ameiurus natalis*
Flat Bullhead	*Ameiurus platycephalus*
Snail Bullhead	*Ameiurus brunneus*
Redbreast Sunfish	*Lepomis auritus*
Green Sunfish	*Lepomis cyanellus*
Redear Sunfish	*Lepomis microlophus*
Bluegill	*Lepomis macrochirus*
Warmouth	*Lepomis gulosus*
Largemouth Bass	*Micropterus salmoides*
Mottled Sculpin	*Cottus bairdi*
Eastern Mosquitofish	*Gambusia holbrooki*

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