Fecundity Estimates for Rainbow Darters, *Etheostoma caeruleum*, in Southwestern Michigan[1]

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ABSTRACT. **Annual fecundity was estimated for female *Etheostoma caeruleum* by counting the number of eggs spawned in aquaria over the breeding season. Previous estimates of annual fecundity for *E. caeruleum* have been based on dissected museum samples where the number of either matures eggs or both mature and immature eggs were counted. In this study, annual fecundity was estimated as 309 eggs (range 180-607 eggs). This value is greater than previous estimates based on counts of only mature eggs but is less than estimates based on counts of both immature and mature eggs. Female *E. caeruleum* most likely spawn multiple clutches but do not ripen and spawn all of their immature eggs. Given that females release an average of eight eggs per spawning, females probably spawn approximately 39 times over the breeding season. This study also considered relationships between standard length, growth, and mean egg mass. Female standard length was inversely correlated with growth and positively correlated with mean egg mass. This suggests that large females may invest more in offspring size and offspring survival relative to their own growth than do small females.**

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INTRODUCTION

Fecundity is an important variable in life-history studies because it is a major component of fitness (Endler 1986, Stearns 1992). Knowledge of the relationships between age-specific life history traits (annual fecundity, egg size, growth, mortality) is vital to understand the role of natural selection and predict population dynamics (Stearns 1992). Despite this, accurate estimates of annual fecundity are lacking for many freshwater fish species due to discrepancies in methodology (Heins and Baker 1993, Parrish and others 1991). Reliable fecundity measures of spawned eggs are sorely needed for many darter species (Gale and Deutsch 1985, Weddle and Burr 1991).

Until recently, fecundity estimates have been based solely on ovarian egg counts obtained from dissections. Counts have been based on either mature eggs or total eggs (both mature and immature eggs) (Winn 1958, Speare 1965, Page 1983, Grady and Bart 1984, Fisher 1990, Orr and Ramsey 1990, Heins and others 1996). Both measures involve critical assumptions concerning the number of clutches females can produce during the breeding season. For fish species that use external fertilization, a clutch is defined as a group of eggs that are ripened and ovulated in the ovary at one time (Heins and Baker 1993). Annual fecundity estimates based on only mature eggs assume that each female spawns one clutch each season. Recent research indicates that females of many species spawn multiple clutches over the breeding season but may not ripen and spawn all of the immature eggs they contain prior to the breeding season (Gale and Deutsch 1985, Weddle and Burr 1991, Heins and Baker 1993, Heins and others 1996). Annual fecundity estimates based only on mature eggs are most likely underestimates of actual fecundity whereas annual fecundity estimates based on both immature and mature eggs may be overestimates.

This paper presents annual fecundity measures (number of eggs spawned in captivity during the breeding season) and size-specific patterns in growth and mean egg mass for the rainbow darter, *Etheostoma caeruleum* Storer from southwest Michigan. *E. caeruleum* is a small (up to 6 cm) bottom dwelling fish that inhabits swift creeks, small rivers, and clear lakes in the Great Lakes and Mississippi River basins in North America (Page and Burr 1991). Spawning takes place from late March/early April to early June (Winn 1958, Hubbs 1985). A female solicits a spawning from a male by performing a nosedig where she forces her head into the gravel and quivers while in an almost vertical position. She then moves her head forward and body down so that her ventral half is buried in the gravel. The male mounts her and vibrates rapidly on her back during which time eggs and sperm are released. The eggs are buried in the gravel and receive no parental care. *E. caeruleum* distributes a clutch of eggs between several spawnings. Females lay approximately 8 eggs per spawn (Fuller unpublished data). Prespawning females typically contain 500-1400 mature and immature eggs depending on age (Winn 1958). The number of eggs spawned during the breeding season has not been previously reported. I present original data on annual fecundity, body size, mean egg dry mass, and adult growth and compare it to previously reported annual fecundity values based on mature and total egg counts.

MATERIALS AND METHODS

In 1996, data were obtained on standard length, annual fecundity, and adult growth rates. *E. caeruleum* was collected with a kickseine from Prairieville Creek, Augusta Creek, and Mill Pond Outlet in Kalamazoo and Barry counties, MI, USA, and returned to Kellogg Biological Station, 2-6 March. Twelve male/female pairs (four pairs from each stream) were matched for similar

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body length, placed in 19-liter aquaria with a rocky substrate, and housed in the laboratory until 17 July 1996. Standard length was measured at the beginning and end of this study. Adult growth was estimated as the difference in standard length between the end and the beginning of the study.

Fish were fed twice daily with a diet of live chironomids and blackworms. Excess food items were always present in the rocks of the aquaria and ensured an adequate food supply. Lights were kept on a 12L:12D ratio in March and April. In May, the photoperiod was adjusted to 14L:10D to approximate natural sunlight patterns. The thermostat in the laboratory was set at 14°C, and water temperatures ranged between 10-15°C for the majority of the breeding season. There were fluctuations in temperatures as aquaria were filled with water from Gull Lake at Kellogg Biological Station every 3-5 days which followed seasonal water temperatures.

Annual fecundity was estimated as the sum of eggs found in an aquarium over the breeding season. Eggs were retrieved during brief egg checks with the use of a vacuum hose once every 3-5 days. Each aquarium was vacuumed once during each egg check and some eggs probably remained in the aquaria. Following egg checks, 3/4 of the water for each aquarium was replaced with freshwater from Gull Lake. Gull Lake supports *E. caeruleum*, so the quality of the aquaria water should have been similar to that found in natural populations.

Two females from August Creek died while in the laboratory. Also, a pair of fish from Prairieville Creek became sick (emaciated with bulging eyes) during confinement. This pair ceased breeding considerably earlier than the other pairs. Data from these individuals were omitted from the analysis resulting in a total sample size of nine.

In 1995, data were obtained on standard length and average egg mass per female. *E. caeruleum* was collected with a kickseine from Prairieville and Seven Mile creeks in Kalamazoo County, MI, USA, and returned to Kellogg Biological Station, March-May. Males and females were held separately in 38-liter aquaria prior to laboratory trials. These animals were held for only a fraction of the 1995 breeding season (2-3 weeks).

To obtain eggs, each female was placed in a 38-liter aquarium with 1-2 males. The standard length of females and males was measured to the nearest mm. Aquaria were observed 2-3 times per day to ascertain whether or not the fish had spawned. If no spawnings took place within 36 hours, then the replicate was canceled.

Eggs were retrieved from aquaria with a vacuum hose, counted, and dried at 36°C for at least 7 days. Dry mass (± 0.001 g) was measured. Trials producing <20 eggs were discarded from the analysis due to the sensitivity of the scale. Average egg mass was calculated by dividing total egg dry mass by the total number of eggs collected for each female.

Statistical analyses were conducted using SYSTAT (Wilkinson and others 1992) statistical package. Significance tests are two-tailed.

RESULTS

Mean annual fecundity was 309.22 ± 44.129 SE eggs per female (n = 9). Eggs were retrieved from aquaria from 14 March-7 July 1996, and all fish had begun spawning by 5 April 1996. Given that females release eight eggs per spawning (Fuller unpublished data), females must spawn an average of 39 times over the breeding season. The peak of spawning occurred between late April and late May, but a few dead eggs (<5) were retrieved from each aquarium from 20 June until 7 July 1996. This is most likely due to the fact that all eggs could not be removed from the aquaria during each check.

Neither female nor male standard lengths differed significantly among populations (females: $F_{2,6} = 2.523$, $p = 0.160$; males: $F_{2,6} = 0.3402$, $p = 0.724$) so populations were pooled in the following analyses (Table 1). There was no significant relationship between female standard length and annual fecundity (Pearson Correlation Coefficient, R = 0.359, n = 9, $p = 0.343$) nor between annual fecundity and growth (R = -0.543, $p = 0.131$, n = 9). Analysis of covariance was used to examine the effects of standard length and population on female growth after testing for homogeneity of slopes. Both population and standard length had significant effects on female growth ($F_{2,5} = 9.183$, $p = 0.0212$; $F_{1,5} = 16.903$, $p = 0.0093$, respectively, Fig. 1). Female growth correlated inversely with original body size (R = -0.862, $p = 0.0028$). Females from Augusta Creek grew significantly less than females from Prairieville Creek (Tukey's HSD test, $p = 0.024$). Male growth was also inversely related to standard length (R = -0.747, $p = 0.0208$, n = 9), but there was no significant effect of population. It is unlikely that differential foraging success accounts for these negative relationships between growth and body size because all fish were fed twice daily and kept in aquaria containing an excess of blackworms. Furthermore, there was no relationship between the growth of males and females paired in the same aquarium as would be expected if different aquaria had drastically different food levels (R = 0.403, $p = 0.282$, n = 9).

Average individual egg mass per female was 0.729 ± 0.025 SE (mg) (n = 19). Standard length did not differ significantly among females from Prairieville ($\bar{x} = 43.20$ ± 0.95 mm) and Seven Mile Creeks ($\bar{x} = 46.75 \pm 1.75$ SE (mm)) (T = -1.724, DF = 17, $p = 0.103$) allowing the two samples to be pooled in subsequent analyses. Analysis of covariance was used to examine the effects of standard length and population on average egg mass after testing for homogeneity of slopes. Average egg mass increased with female standard length (Fig. 2, R = 0.537, n = 19, $p = 0.0179$). However, population had no effect upon average egg mass of females ($F_{1,16} = 0.0001$, $p = 0.992$; Prairieville Creek: $\bar{x} = 0.718 \pm 0.031$ SE (mg); Seven Mile Creek: $\bar{x} = 0.772 \pm 0.027$ SE (mg)). There was no relationship between date and egg mass (R = -0.143, n = 19, $p = 0.537$).

DISCUSSION

These data have important implications for measuring annual fecundity in darters. Average annual fecundity

TABLE 1

Population, standard length (mm), growth (Δ standard length over the breeding season (mm)), and annual fecundity (number of eggs spawned in captivity during the breeding season) for each male/female pair.

Population	Female Standard Length (mm)	Male Standard Length (mm)	Female Growth (Δmm)	Male Growth (Δmm)	Annual Fecundity
Augusta Creek	56.0	46.0	0.0	3.5	269
Augusta Creek	54.0	51.0	1.0	2.0	607
Mill Pond Outlet	50.0	50.0	3.0	6.0	330
Mill Pond Outlet	46.0	45.0	3.0	6.0	415
Mill Pond Outlet	44.0	44.0	4.0	4.0	225
Mill Pond Outlet	39.0	43.0	6.0	5.0	301
Prairieville Creek	54.0	54.0	4.0	2.0	203
Prairieville Creek	44.0	50.0	5.5	3.0	253
Prairieville Creek	39.0	40.0	6.0	8.0	180
Mean (SE)	47.3 (2.15)	47.0 (1.50)	3.61 (.706)	4.39 (.673)	309 (44.1)

was 309 eggs per female. Previous studies have estimated annual fecundity by counting either mature and immature eggs or only mature eggs. Counts of mature and immature eggs contained within females in another Michigan population estimated annual fecundity at 508-1462 eggs (Winn 1958). In this study, annual fecundity ranged from 180 to 607, considerably lower values than those reported by Winn indicating that counts of immature and mature eggs are overestimates of annual fecundity. In contrast, counts of only mature eggs tend to underestimate annual fecundity. Grady and Bart (1984) reported that the number of mature eggs in 19 females collected in Louisiana in March, April, and June ranged from 17-125 eggs with a mean of 66 eggs per female. A similar study in Mississippi measured clutch size (counts of mature eggs) as 14-60 eggs (Heins and others 1996). The present study did not measure clutch size. However, the fact that the annual fecundity estimates obtained in this study were five times larger than clutch size estimates (Grady and Bart 1984, Heins and others 1996) indicates that *E. caeruleum* produces multiple clutches over the breeding season. As has been found in other darters, counts of mature eggs from dissections are not accurate measures of annual fecundity in *E. caeruleum* (Gale and Deutsch 1985, Weddle and Burr 1991).

A problem inherent to obtaining annual fecundity estimates from captive fish is that the laboratory may not sufficiently replicate the natural breeding environment. I attempted to create as realistic an environment as possible. Light/dark ratios were adjusted to follow seasonal

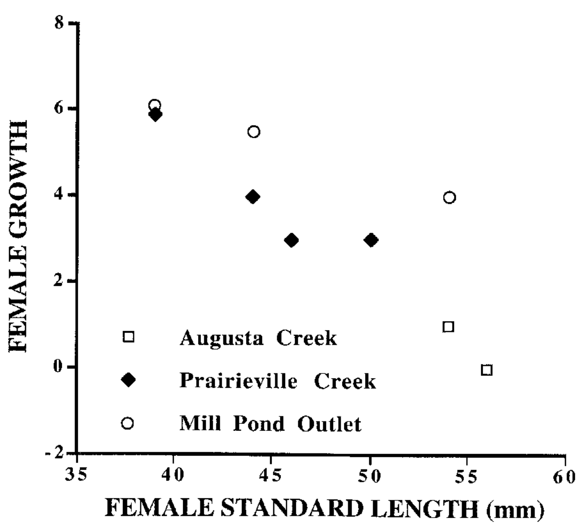

FIGURE 1. The relationship between female growth (Δmm) and female standard length (mm) for females from 3 populations. Female growth is measured as the change in standard length (Δmm) from the beginning to the end of the breeding season. Overlapping data points are jittered for graphical presentation. N = 9.

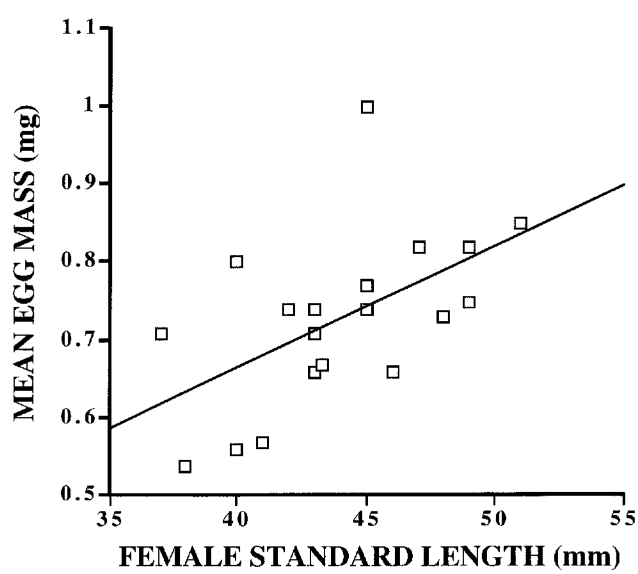

FIGURE 2. The relationship between mean egg mass (mg) and female standard length (mm). N = 19.

patterns. Aquaria water was obtained from a lake containing *E. caeruleum*. Live food was fed to all fish. Stream temperatures were not monitored, and it is unknown whether lab water temperatures and stream water temperatures were similar. If water temperatures in aquaria were higher than stream water temperatures, then this may have slightly decreased inter-clutch intervals or increased egg production and raised annual fecundity (Gale and Deutsch 1985, Weddle and Burr 1991). In contrast, constant close proximity of parental fish to eggs in aquaria may have led to egg cannibalism that reduced the annual fecundity estimate. Similarly, if fish were stressed due to confinement in aquaria, then annual fecundity may have been unnaturally lowered. Such problems are inherent to any study manipulating animals. The fact that the spawning behavior of aquaria fish was similar to that reported for stream fish indicates that the laboratory environment was sufficient. Winn (1958) reports *E. caeruleum* spawning as early as 22 March. In this study, one pair had spawned by 14 March, but some of the fish did not spawn until 5 April. In addition, the largest numbers of eggs were obtained from late April–late May which is the same time as the peak in spawning activity in streams. These measurements of annual fecundity for *E. caeruleum* are currently the most accurate measurements available because they are direct counts of spawned eggs.

This study also shows an interesting pattern between size-specific growth and mean egg mass. Large females laid heavier eggs but grew less than small females. Large egg mass most likely has strong affects upon offspring survival. In the closely related orangethroat darter, *E. spectabile*, offspring from large eggs are larger at hatching and less susceptible to starvation than offspring from small eggs (Marsh 1986). The finding that large females grow less than small females is most likely accounted for by age differences in growth. Growth diminishes with age in both sexes (Lutterbie 1979, Grady and Bart 1984). Young, small females may allocate less energy into egg mass and more into adult growth than old, large females.

In conclusion, this study found that female adult darters spawned an average of 309 eggs (range 180-607) over one breeding season. This value is greater than previous estimates based only on counts of mature eggs but is less than estimates based on counts of both immature and mature eggs. Female *E. caeruleum* most likely spawn multiple clutches but do not ripen and spawn all of their immature eggs. Also, female standard length is inversely correlated with growth and positively correlated with mean egg mass. This suggests that large females may invest more in offspring size and offspring survival relative to their own growth than do small females.

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