REPRODUCTION, LIFE HISTORY, AND DIETS OF THE GREENTHROAT DARTER *ETHEOSTOMA LEPIDUM* IN LOW-FLOW AND HIGH-FLOW ENVIRONMENTS

by

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ABSTRACT

The Greenthroat Darter *Etheostoma lepidum* (Class Actinopterygii) is a member of the *Austroperca* complex, along with five other darters found primarily in the Rio Grande drainage of USA and Mexico. Greenthroat Darter, however, is the most widely distributed *Austroperca* and found east of the Rio Grande drainage into the Edwards Plateau region of central Texas. Threats, as with other aquatic organisms found in arid and semi-arid environments, include natural and anthropogenic modifications to stream flow and water permanency. Purpose of this study was to gain a better understanding of the mechanisms related to low flows and population viability of stream fishes. Using Greenthroat Darters as a representative of the understudied Austroperca complex, study objectives were to quantify life history traits, reproduction, and diets of the Greenthroat Darter and to assess the effects of low flow on their reproduction and diets. Greenthroat Darters were sampled monthly for one year from two environments within the Comal River (Comal County, TX): a low-flow environment, where stream flows were reduced because of a downstream dam and consisted of a persistent low-flowing (0.1 m3/s) pool mesohabitat; and a high-flow environment, where stream flows are sufficient (2.6 m3/s) to maintain a typical riffle mesohabitat for Greenthroat Darters. Study results demonstrated Greenthroat Darters had an 11-month spawning season from October through August, produced multiple batches of ova during the spawning season, lived up to 2 years, and consumed primarily aquatic insects. Differences between flow environments consisted of greater parasite prevalence, lower fish condition, and fewer

food items consumed in the low flow environment compared to the high flow environment. However, energy invested into reproduction (i.e., gonadosomatic index, batch fecundity) was not detected between the low flow and high flow environments. Despite greater number of parasites and lower condition, Greenthroat Darters have persisted in the low flow environment since the construction of the dam (late 1800s). Therefore, there is uncertainty among the linkages between typical measures of fish health (e.g., number of parasites, condition) and population viability.

1. INTRODUCTION

The Greenthroat Darter *Etheostoma lepidum* is a member of the monophyletic sub-group *Austroperca* (Percidae; subgenus *Oligocephalus*), along with the Rio Grande Darter *E. grahami*, Conchos Darter *E. australe*, Rio Salado Darter *E. segrex*, Mexican Darter *E. pottsii*, and Tufa Darter *E. lugoi* (Near et al. 2011). Members of *Austroperca* are endemic to the semi-arid and arid Rio Grande drainage of USA and Mexico (Norris and Minckley 1997, Kuehne and Barbour 2015), except for the Mexican Darter (i.e., found west of the Rio Grande drainage; Norris and Minckley 1997) and the Greenthroat Darter (i.e., found east of the Rio Grande drainage; Craig and Bonner 2019). Within their range, members of *Austroperca* generally associate with flowing water habitats in spring-fed tributaries (Contreras-Balderas 1977, Page 1983, Kuehne and Barbour 1983, Norris and Minckley 1997).

Spring-fed tributaries in semi-arid and arid climates are often described as aquatic evolutionary refugia (Keppel et al. 2012). Groundwater and spring outflows tend to provide greater water permanency in semi-arid and arid climates, decoupling water availability from low amounts of precipitation over long periods of time (e.g., interglacial periods). With greater water permanency, relic and subsequently endemic fauna persist within small geographical regions (Davis et al. 2013). Evolutionary Refugia Concept is proposed as the mechanisms to explain the large number of endemic fishes, including Rio Grande Darter and Greenthroat Darter, within the semi-arid and arid southwestern USA (Craig et al. 2016). Currently, many of the endemic fishes associate strongly with the spring habitats, specifically the thermally stable water temperatures of the spring outflows (Hubbs 1995). Fish association with spring habitats and thermally stable water temperatures is linked to temperature-mediated performance (Craig et al. 2019). An additional commonalty among spring-associated fishes is a protracted spawning season, being reproductively active for a longer period than fish not found in springs (Hubbs 1985, Folb 2010, Robertson et al. 2016). Relatedly, population numbers and reproduction are estimated to be linked to the amount of spring flow, with population numbers and reproduction decreasing under low-flow conditions (Mora et al. 2013). Collectively, many of the spring-associated fishes in southwestern USA are listed as species of conservation concern (Faucheux et al. 2019), given their associations with a limited number of spring-fed tributaries in semi-arid and arid areas.

Information is limited on the life history characteristics, reproductive patterns, and trophic guilds for members of *Austroperca*. Maximum length ranges between 38 mm in Tufa Darter (Norris and Minckley 1997) to 64 mm in Greenthroat Darter (Kuehne and Barbour 1983). Spawning season is reported to be during Spring and early Summer for Rio Grande Darter (Harrell 1980), Conchos Darter (Page 1983), Mexican Darter (Meek 1904) and from Fall through Spring for the Greenthroat Darter (Hubbs et al. 1968). Eggs are demersal and adhesive (Aguilera et al. 1999) and attached to vegetation or rock substrates in Greenthroat Darter (Strawn 1955) and Rio Grande Darter (Strawn 1956) or on rock substrates in Conchos Darter (Meek 1904, Page 1983). Eggs are released in multiple batches (e.g., up to 13 batches in Greenthroat Darter, Strawn 1956) and up to 100 per batch (Strawn 1956). For diet, Conchos Darter and Mexican Darter are reported to consume insects (Contreras-Balderas 1977, Page 1983). Among the *Austroperca* group, more is known about the Greenthroat Darter than other members of the group; however, the available information is based on laboratory observations from fish taken from the wild but otherwise limited on the specifics of age and growth, ovarian cycles, and diet. The wider distribution of the Greenthroat Darter enables more opportunities to use this species as a representative of the *Austroperca* group in assessing more detailed life history traits, reproduction patterns, trophic guilds, and the influence of spring flows on reproduction.

Purposes of this study are to describe life history traits, reproduction, and diets of the Greenthroat Darter, taken monthly from two sites on the Comal River (Comal County, Texas) for a period of one year. The two sites (Upper Spring Run, flow: 0.1 m^3/s; New Channel, flow: 2.6 m^3/s) reflect different flow environments to assess the influence of flow on life history traits, reproduction, and feeding of the Greenthroat Darter. Study objectives were to 1) quantify number of age groups, life span, and growth of the Greenthroat Darter, 2) calculate monthly gonadosomatic index (GSI) and quantify ovary and oocyte stages of female Greenthroat Darters, and 3) assess monthly stomach contents, documenting frequency of occurrence and relative proportion of food items and, if present, parasites in the gastrointestinal tract of Greenthroat Darters. Predictions are that Greenthroat Darter populations will consist of three age groups with a maximum life span of up to two years based on the number of age groups and life span of other members of subgenus *Oligocephalus* (e.g., Orangethroat Darter *Etheostoma spectabile*, Edwards 1997), spawning season will be longer than the five months observed by Hubbs (1961) since spring-associated fishes typically have spawning seasons ranging from 9 to 11 months (Folb, 2010, Perkin et al. 2012, Robertson et al. 2016), and that diets will consist of aquatic insects, based on the diets reported for the Conchos Darter and the Mexican Darter (Contreras-Balderas 1977, Page 1983) and cladocerans, copepods, amphipods, and aquatic insects, based on diets of the Fountain Darter *E. fonticola* taken previously from the Comal River (Bergin et al. 1996). Per Mora et al. (2013) model estimation that flows <1.4 m^3/s in the San Marcos River reduces Fountain Darter reproduction, I predict that Greenthroat Darter reproduction (i.e., GSI, ova counts), a riffle specialist which tends to be more suspectable to reductions in flow (Aadland 2011) than slackwater species like the Fountain Darter, will be less in the low-flow environment (0.1 m^3/s) than in the high flow environment than in the high-flow environment (2.6 m^3/s). Additionally, low-flow conditions can affect feeding and parasite loads of fishes (Hockley et al. 2014); therefore, I predict that diets in the low-flow environment will differ (e.g., less diversity of food items, greater number of empty stomachs) from those in the high-flow environment and that parasite load (e.g., parasite diversity and numbers) will be greater in individuals taken from the low-flow environment than those from the high-flow environment.

2. METHODS

Study Area

Comal River originates from multiple spring outflows of the Edwards Aquifer in Comal County, Texas, and flows 6 km before merging with the Guadalupe River (Brune 1981). The original spring complex was modified with low-head dam, diversion channel, and bank retaining walls. The low-flow environment was located in Upper Spring Run (29.720361, -98.128583) of Landa Lake. Under average precipitation, mean flows (± 1 SD) are 0.1 (0.09 m^3/s) (Nichols 2015), but subject to high flows during localized rainfall events. Habitats within the low-flow environment are influenced by a dam that creates Landa Lake; hence water depths (mean: 1.03 m, SD: 0.39, max: 2.3 m; 2014 – 2020, T. Bonner, unpublished data) are artificially deeper than pre-impoundment depths, and current velocities (mean: 0.05 m/s, SD: 0.59, max: 0.80 m/s) are artificially slower than pre-impoundment current velocities. Substrates consist primarily of gravel (42%), followed by silt (24%) and cobble (16%). Mean vegetation cover is 46% with vegetation predominantly consisting of filamentous algae (32%), bryophytes (20%), detrital algae (8%), and *Cabomba* (5%). The high-flow environment was located immediately downstream from Landa Lake dam outflow in the area called New Channel (29°42'21.0"N 98°07'51.9"W). Mean daily flow (± 1 SD) was 2.6 (2.09) m^3/s between 2014 and 2020 (USGS Station 08168932). Habitats within the high-flow environment are influenced by Landa Lake outflow, specifically the swift currents (mean: 0.40 m/s, SD: 0.25, max: 1.10 m/s) created by a 4 m waterfall from Landa Lake dam to form the beginning of the New Channel. Mean depth (± 1 SD) is 0.73 (± 0.28; max: 1.37) m (2014 – 2020, T. Bonner, unpublished data). Substrates consist primarily of gravel (49%), followed by cobble (20%), and sand (13%). Mean vegetation cover is 36% with vegetation predominantly consisting of *Vallisneria* (16%), *Justicia* (14%), and *Ludwigia* (10%). Since both sites were within close proximity to spring outflows (Groeger et al 1997), mean and variability of water quality parameters were similar between sites. Mean water temperature (± 1 SD) was 23.6 (0.61) °C at the low-flow environment and 23.3 (0.80) °C at the high-flow environment, mean dissolved oxygen was 7.4 (2.23) mg/L at the low-flow environment and 8.4 (0.88) mg/L at the high-flow environment, and specific conductance was 570 (12) µS/cm at the low-flow environment and 534 (137) µS/cm at the high-flow environment.

Field Collections and Laboratory Processing

Fish were collected monthly, during the second or third week of each month, for twelve consecutive months (November 2019 - October 2020). For each collection and site, a seine net (3 m x 1.8 m; 3.2 mm mesh size) was used to collect specimens. Total length (TL), to the nearest mm, was measured on all Greenthroat Darters collected. Up to 8 female Greenthroat Darters, identified by light dorsal fin coloration and lack of green color on isthmus region, were retained per collection and site. However, sexually immature males can have light dorsal fin coloration and lack of green color on isthmus region and were inadvertently collected at times. Greenthroat Darters were anesthetized in a lethal dose of Tricaine Methanesulfonate (MS-222; >80 mg/l), fixed in 10% formalin for two weeks, rinsed, and then transferred to 70% ethanol.

In the laboratory, Greenthroat Darters were removed from 70% ethanol solution, blotted dry, measured (TL), and weighed to the nearest mg. An incision was made in the abdomen region from the urogenital opening to isthmus. The esophagus was severed and viscera with gonads were removed from the body cavity. Fish were reweighed to obtain an eviscerated weight. Males and females were identified based on the presence of testes or ovaries. Testes were not processed any further, but stomach contents of males were included in stomach analysis. Ovaries were separated from the viscera. Ovaries were next examined under light microscopy. Stage of ovarian development (i.e., latent, early developing, late developing, spawning, and spent) were identified based on size and color of oocytes and ova (Nichols 2015) and weighed to the nearest mg. One spawning ovary per month, excluding September, was dissected to count the ova. If both ovaries were about equal in size (i.e., one ovary nearly the same length as the other ovary), oocytes were removed from the slightly larger ovary. If the two ovaries were asymmetrical in length (i.e., one ovary <50% of the length than the other ovary; N = 10 females), both ovaries were processed together for oocyte diameters and counts of late vitellogenic oocytes and ova (Nichols 2015). For each female, the ovary or ovaries were placed in a glass dish, and the oocytes and ova were teased apart. Oocytes and ova were redistributed within the dish with gentle swirling An ocular on a microscope was caliberated with a stage micrometer measuring 1 mm, for the first 100 oocytes in field of view, oocyte diameters were measured to the nearest 0.01 mm with ocular micrometer. The number of ova (proved recognizable via the ovum indentation and oil droplet; Nichols 2015) were enumerated. The number of late vitellogenic oocytes and ova were doubled to estimate batch fecundity per individual for those with only one ovary examined. The stomachs of males and females were removed from the viscera by severing the small intestine. The stomach was weighed to the nearest mg. A longitudinal incision was made along the stomach to expose the contents. Volume of contents (i.e., the amount of the stomach filled by food contents; stomach fullness) was estimated visually by two observers from 0 to 100% in increments of 10% (Childs et al. 1998) the two estimates were then averaged and recorded. Individual items were grouped by food item categories (i.e., family-level for aquatic insects, non-insect invertebrates, and algae). Food item categories were weighed individually when practical. The number of parasites in the stomach and viscera were identified to the lowest practical taxonomic level and enumerated.

Data Analyses

Length frequency histograms were constructed from collections of all Greenthroat Darters by using 2-mm bin increments that were combined across both sites, regardless of sex, to estimate the number of age groups by month, and overall life span. Modal progression analysis (Bhattacharya's Method *in* Fish Stock Assessment Tools II [FiSAT II]; Gayanilo et al. 2005) was used to estimate the number and total length of age groups (Perkin et al. 2012, 2013) between November 2019 and October 2020. Gonadosomatic index ([mass of ovary/mass of eviscerated fish] * 100) was calculated for each individual. Mean GSI was calculated by site and month as well as the combined sites by month. Months with elevated GSIs were indicative of reproductive season (i.e., period of yolk deposition into the ovaries). Monthly proportions of latent, early developing, late developing, spawning, and spent ovaries were overlayed with GSIs. Months with spawning ovaries (late developing and spawning) were indicative of spawning season (i.e., occurrence of ovum). The relationship between monthly mean GSI and percent of spawning ovaries were assessed with correlation analysis. Mean number of food items consumed, mean weight of food items consumed, and mean parasite counts were compared between environments with one-factor ANOVAs. Condition factor (weight/TL^3*100) was also calculated for each fish as a measure of fitness.

Gonadosomatic indices during reproductive season, number of oocytes during reproductive seasons, and number of parasites between the low-flow environment and the high-flow environment were assessed individually with one-factor ANOVAs.

Percent occurrence, mean percent by number, and mean percent by weight of each food item were calculated (Bowen 1996). Percent occurrence was calculated as the proportion of fish with a food item multiplied by 100. Mean percent by number was calculated as the mean in the number of each food item across all individuals (overall and by site), summed the mean of all food item numbers, and divided the mean of each food item number by the sum of all food item mean counts multiplied by 100. Mean percent by weight was calculated as the mean in the weight of each food item across all individuals (overall and by site), summed the means of all food item weights, and divided the mean of each food item weight by the sum of all food item mean weights multiplied by 100.

3. RESULTS

Total lengths were measured from 308 male and female Greenthroat Darters captured from the two flow environments. Monthly modality in mean lengths (± 1 SE) estimated with FiSAT 1.2 indicated two age groups (ages 0 and 1) in 2019 and two age groups (ages 0 and 1) in 2020 (Figure 1). Estimated life span was about 1.5 years. First detection of age 0 fish in 2020 was in April.

Among the 308 Greenthroat Darters captured, 119 female Greenthroat Darters and 14 males Greenthroat Darters were taken. Among females, gonadosomatic indices were elevated (> 2%) from November 2019 through May 2020 and again in October 2020 (Figure 2 top). Latent ovarian stage was observed in November and December and from April to September (Figure 2 bottom). Early developing ovarian stage was observed from November to January, March, and from June to October. Late developing ovarian stage was observed in all months except June and July. Spawning ovarian stage was observed in all months except September. Monthly percentages of spawning ovaries were positively correlated ($r = 0.70$, $N = 12$, $P < 0.01$) with monthly mean GSIs. Batch fecundity was estimated from 36 females with spawning ovaries. Mean (± 1 SE) batch fecundity among both sites was 31.2 ± 4.46. Among females ranging in total length from 36 to 62 mm with spawning ovaries in all months except September, multiple modes were observed monthly in diameter-frequency distributions (Figure 3).

Diet assessments were made on 119 female Greenthroat Darters and 14 male Greenthroat Darters. Percent of empty stomachs was 8.2% (N = 11). Among 122 Greenthroat Darters with at least one food item in their stomach, mean (± 1 SE) percent stomach volume of food items was 63% ± 2.8. Between both flow environments, Greenthroat Darters consumed 20 taxonomic groups of prey items. The most frequently occurring item was Amphipods (41% in percent occurrence), followed by Baetidae (37%), and Chironomidae (29%) (Table 1). The most abundant per mean percentage by number was Hydropsychidae (29%) followed by Amphipods (20%), Baetidae (15%), and Chironomidae (14%). The most abundant per mean percentage by weight was Baetidae (38%) and Amphipods (21%).

Comparisons Between Low-Flow and High-Flow Environments

Among the 308 male and female fish captured, 126 Greenthroat Darters (range: 10 – 60 mm TL) were captured from the low-flow environment, and 182 Greenthroat Darters (range: 29 – 67 mm TL) were captured from the high-flow environment. Age-0 fish composed 30% of the population in the low-flow environment and 26% of the population in the high-flow environment across months. However, lower proportions of age-0 fish were observed monthly from April through October (30-100%) in the low-flow environment than in the high-flow environment (71-100%) (Table 2). Correspondingly, female GSI was greater ($F_{1,118}$ = 2.3; P = 0.14) in the low-flow environment (mean GSI: 2.9; 1 SE: 0.28; N: 58) than in the high-flow environment (mean GSI: 2.3; 1 SE: 0.33; N: 62), most notably during the months of April through October (Figure 4 and 5). Excluding age-0 fish, age-1 fish GSI with late developing and spawning ovaries was not different ($F_{1,29}$ = 1.8; P = 0.19) in the low-flow environment (mean GSI 3.8; 1 SE: 0.41; N: 18) than in the high-flow environment (mean GSI 5.0; 1 SE: 0.93; N: 13) (Figure 6 top). Likewise, batch fecundity was not different ($F_{1,34}$ = 0.32; P = 0.58) in the low-flow environment (mean batch fecundity: 29.3; 1 SE: 5.1; N: 23) than in the high-flow environment (mean batch fecundity: 34.5; 1 SE: 8.6; N: 13) (Figure 6 bottom).

Among the 133 Greenthroat Darters (119 females and 14 males) taken for diet assessments, 65 fish (range: 21 to 57 mm TL) were taken from the low-flow environment, and 68 fish (range: 31 to 65 mm TL) were taken from the high-flow environment. Percentages of empty stomachs were 7.7% in the low-flow environment and 8.8% in the high-flow environment. Among fish with at least one food item in their stomach, mean (± 1 SE) percent gut fullness was 66% ± 3.7 in the low-flow environment and 59% ± 4.2 in the high-flow environment. Number of diet items per individual differed ($F_{1,131} = 4.9$; $P = 0.03$) between the low-flow environment (mean number of food items consumed per individual: 4.6 items; 1 SE: 0.54; N: 65) and the high-flow environment (mean: 8.35; 1 SE: 1.54; N: 68). However, weight of diet items was not different ($F_{1,131} = 0.01$; $P = 0.93$) between the low-flow environment (mean weight of food items consumed per individual: 2.00; 1 SE: 0.26; N: 65) and high-flow environment (mean: 1.96; 1 SE: 0.31; N: 68). At the low-flow environment, the most frequently occurring item was Amphipods (60% in percent occurrence), followed by Baetidae (29%), and Chironomidae (23%) (Table 3). The most abundant per mean percentage by number was Amphipoda (51%), followed by Baetidae (11%), and Hydroptilidae (10%). The most abundant per mean percentage by weight was Amphipoda (32%), followed by Baetidae (30%), and Ephemeroptera (10%). At the high-flow environment, the most frequently occurring item was Hydropsychidae (47% in percent occurrence), followed by Baetidae (46%), and Chironomidae (35%) (Table 4). The most abundant per mean percentage by number was Hydropsychidae (45%), followed by Baetidae (17%), and Chironomidae (17%). The most abundant per mean percentage by weight was Baetidae (47%), followed by Hydropsychidae (13%), and Amphipods (10%).

Fish taken from the low-flow environment were noticeably emaciated compared to the fish taken from the high-flow environment. The relationship between condition factor and TL differed (ANCOVA; interaction term; $F_{1,132} = 6.6$; $P = 0.01$) between low-flow environment and high-flow environment with a mean (± 1 SE) condition factor across all lengths of 0.83 (± 0.12) at the low-flow environment and 0.98 (± 0.02) at the high-flow environment (Figure 7). In addition, the number of endoparasites were greater ($F_{1,131} = 10.5$; $P = 0.001$) in the low-flow environment (mean count: 7.4; SE: 1.55) than in the high-flow environment (mean count: 2.2; SE: 0.55) (Figure 8). Endoparasite *Leptorhynchoide* (Phylum Acanthocephala) was the more abundant parasite comprising 98%, whereas as *Camallanus* (Phylum Nematoda) comprised 2% of the endoparasite community. *Leptorhynchoide* were found in the intestine and ovaries. *Camallanus* were found in the intestine.

4. DISCUSSION

Study results supported several of the initial predictions about Greenthroat Darter longevity, protracted spawning season, and diets. Greenthroat Darters had an 11-month spawning season, which is greater than the five-month spawning season reported by Hubbs 1961, produced multiple batches of ova within the spawning season, and consumed primarily aquatic insects. Between flow environments, Greenthroat Darters consumed fewer amounts of food items and had greater parasite loads in the low-flow environments than those in the high-flow environment. Among predictions not supported by the study results, the two-year life span of Greenthroat Darters reported in this study was less than the three-year life span of other members of subgenus *Oligocephalus* (e.g., Orangethroat Darter *Etheostoma spectabile*, Edwards 1997). Measures of reproduction (i.e., GSIs, batch fecundity) were not different among females of reproductive size between low- and high-flow environments as predicted for another darter species, which is a slackwater specialist within the Comal River (Mora et al. 2013). A noteworthy result that was not part of our initial predictions was the presence of spawning ovaries in estimated age-0 fish within the high-flow environment in August 2020, suggesting that females become sexually mature within their first year (i.e., age 0).

Study results indicated that Greenthroat Darters invested energy into reproduction all year with spawning during an 11-month period. The 11-month spawning period reported herein is longer than the five months previously reported by Hubbs (1961) in a tributary of the Colorado River (water temperature not reported). Differences between the two studies might illustrate habitat-mediated influences on fish reproduction and therefore plasticity in reproductive season length. Temperate fishes generally rely on water temperature and photoperiod as cues for reproductive cycles (Wang et al. 2010).

Members of the genus *Etheostoma* typically spawn during the Spring and Summer for about three to four months (Page 1983). However, fishes in thermally stable water temperatures, such as the Comal River, have protracted spawning season. Fountain Darter spawning season, taken from thermally stable waters of the Comal River and nearby San Marcos River, is identical to that of Greenthroat Darter with an 11-month period and not in September (Nichols 2015). Guadalupe Darter *Percina apristis*, taken from the thermally stable waters of the San Marcos River, spawns for 11 months and not in July (Folb 2010). Also, within the thermally stable waters of the San Marcos River, Ironcolor Shiner *Notropis chalybaeus* spawns for 10 months (i.e., March through December), which is four months longer than conspecifics from non-thermally stable waters (Perkin et al. 2012). Extending spawning season length, as demonstrated by the Greenthroat population in this study from those reported by Hubbs (1961) could be another example of plasticity in reproductive timing and length of spawning efforts. Hubbs (1985) proposed that warmer temperatures ceased Spring or Summer spawning of darters in non-thermally stable waters. Therefore, lack of warmer temperatures in thermally stable systems might eliminate proximate cues for gonadal quiescence, allowing a phylogenetically diverse community of fishes to extend the length of their spawning season. Although spawning seasons are extended, it is unclear as to the cues for gonadal quiescence or cessation of spawning given that one or two months with reduction in gonadal investment or without spawning is common among the darters in Summer and early Fall (this study, Folb 2010, Nichols 2015) and among minnows in late Fall and Winter (Perkin et al. 2012, Robertson et al. 2016, Craig et al. 2017).

Per the diet items quantified, the Greenthroat Darter is consistent with the benthic invertivore feeding guild as described by Goldstein and Simon (1999) and consistent with the main dietary items consumed by most darters (Page 1983). The diversity and amounts of food items consumed by the Greenthroat Darter were similar to those reported for other members of *Austroperca* (i.e., aquatic insects; Contreras-Balderas 1977, Page 1983) and to the co-occurring Fountain Darter. Among larger sized (>31 mm in TL) individuals quantified by Bergin (1996), Fountain Darters consumed, in order of selectivity indices, amphipods, ephemeropterans, dipterans, cladocerans, and copepods. Thus, Greenthroat Darters and Fountain Darters have overlap in prey items consumed.

Greenthroat Darters consumed fewer diet items overall, fewer Hydropsychidae and Baetidae, and greater number of Amphipoda (*Hyalella*) in the low-flow environment than in the high-flow environment. However, diet weight, percent gut fullness, and percent of empty stomachs were similar. Differences in diets were attributed largely to the association of prey items within each flow environment. Amphipoda *Gammarus pulex* tends to be more abundant in pools than riffles (Dahl and Greenberg 1996) and other *Gammarus* tend to be associated with slow or sluggish flows or standing waters (Extence et al. 1999). In contrast, eight of the nine species of *Hydropsyche* assessed by Extence et al. (1999) were associated with moderate or fast flows or with rapid flows, and all nine of the species of *Baetis* were associated with moderate to fast group.

Differences in observed parasite loads between the low- and high-flow environment were attributed to diet differences, and therefore, the associations of prey items within each flow environment. Endoparasite *Leptorhynchoide* (Phylum Acanthocephala) intermediate host is Amphopoda (*Hyalella*), which were consumed in greater frequency, number, and weight in the low-flow environment than in the high-flow environment. Our initial prediction that greater number of parasites would be found in the low-flow environment than in the high-flow environment was based upon the concept that prevalence of parasites is related to degradation of the aquatic systems (Artim et al. 2019), like the low-flow environment in this study due to impoundment, and inversely related to current velocity (Hallett & Bartholomew 2007). However, prevalence of parasites related to habitat degradation depends on the specificity of parasite and host. Host generalists tend to increase with habitat degradation, whereas host specialists, like endoparasite *Leptorhynchoide* (Phylum Acanthocephala) can be independent of levels of habitat degradation (Artim et al. 2019). Nevertheless, I tentatively attributed the emaciated appearance and low condition factors of Greenthroat Darters in the low-flow environment to the high prevalence of the endoparasites, given that diet weight, percent empty stomachs, and percent gut fullness were similar between fish in the low-flow environment compared to the high-flow environment. Alternatively, the emaciated appearance in the low flow environment could be related to phenotypic variation (i.e., shallower body) associated with low flow environment (Franssen et al. 2013), although shallower body (e.g., more streamlined body) is typically associated with high flow environments (Langerhans 2008). Nevertheless, the perceived emaciated appearance could be attributed to another selection pressure as synthesized by Franssen et al. (2013), such as predator densities differences between the low flow and high flow environments with more elongated bodies found in fishes (i.e., Poeciliidae) with predators than in predator-free populations (Langerhans et al. 2004).

Unlike model estimations of Fountain Darter reproduction decreasing at flows <1.4 m^3/s (Mora et al. 2013), reproductive effort as defined herein (i.e., GSI, batch fecundity) were similar between the low- and high-flow environment. This result is surprising given that riffle specialists (i.e., swift current velocity habitat), like the Greenthroat Darter (Hubbs et al. 1953), would be more sensitive to differences in low-and high-flow environments than the more slackwater-associated species (Aadland 2011), like the Fountain Darter (Behen 2013). Other riffle specialists, such as the Orangethroat Darter (Simon and Wallus 2006) and Slenderhead Darter *Percina phoxocephala*, Thompson 1980), have higher abundances in flowing waters and will move from areas with lower flows, such as habitats influenced by low head dams, to higher flows (Tiemann et al. 2004). Movement to swifter current velocities is thought to be a preference for larger substrates, which in turns increases survival of the individuals. However, Greenthroat Darters in the low flow environment do have access to swifter current velocities and higher flows, both within about 3 km from their current location. Why they remain in the low-flow environment is unknown at this time, but their occurrence in the low-flow environment has likely persisted since the construction of the various dams that formed the impoundment since the late 1800s, including low-flow periods of the 1950s when Fountain Darters were possibly extirpated from the Comal River (Schenck and Whiteside 1976). It should be noted, however, that Mora et al. (2013) models included estimates of age-0 fish survival and recruitment to sexual maturity. Age-0 fish survival and recruitment were not quantified in this study.

Greenthroat Darters and other members of *Austroperca* are associated with spring-fed systems in semi-arid and arid regions. Descriptions of life-histories and understanding how modifications from high-flow environments to low-flow environments (e.g., low head dams, groundwater pumping, diversions of surface flows) will benefit efforts in managing water quantity (i.e., flow) (Gore and Nestler 1988, Jowett 1997) in addition to managing water quality (i.e., Clean Water Act of 1972). A challenge to resolve is the disconnect between aquatic organism's relationship to current velocity (m/s) and flow (m^3/s). Flow (or discharge) is the commodity sold to municipalities, agriculture, and industries with most rivers in Texas having instream flow standards (e.g., Vaugh et al. 2011), whereas current velocity is the measure often associated with fish occurrence and abundances (Mattingly et al. 2003, Henry & Grossman 2008, Sterling & Warren 2017). Relating fish fitness to flow rather than current velocity, as in this study, will enable a direct link between instream flow standards and fish community responses.

Table 1. Percent (%) occurrence, mean percent number of each food item, and mean percent weight of each food item in the stomachs of 122 Greenthroat Darters *Etheostoma lepidum* taken from the Comal River, November 2019-October 2020.

	% occurrence	Mean % by number	Mean % by weight
Coleoptera			
Elmidae	7.5	1.6	<0.1
Psephenidae	3.7	0.6	<0.1
Diptera			
Chironomidae	29	14	1.7
Culicidae	0.7	0.1	<0.1
Stratiomyidae	0.7	0.1	1.2
Thaumaleidae	0.7	0.1	<0.1
Unidentified	1.5	0.2	0.8
Ephemeroptera			
Baetidae	37	15	38
Leptohyphidae	6	1.3	5
Leptophlebiidae	3	0.5	1
Unidentified	13	4	7.6
Trichoptera			
Hydropsychidae	24	29	6.8
Hydroptilidae	23	7.5	7.2
Philopotamidae	0.7	0.1	1.3
Unidentified	5.2	1.6	0.8
Unidentified insects	24	0.2	4.3
Non-insects			
Amphipoda	41	20	21
Cladocera	8.2	3.1	<0.1
Decapoda	0.7	0.1	2
Tricladida	0.7	0.1	0.5
Algae	5.9		0.3

Table 2. Total number of fish collected from the low-flow environment and the high-flow environment by month, separated by their estimated ages and taken from the Comal River, November 2019-October 2020.

Flow environment	Month	N of fish	Age-0	Age-1	Percent of age-0 fish
Low	Nov	12	12	0	100
	Dec	5	5	0	100
	Jan	25	0	25	0
	Feb	10	0	10	0
	Mar	10	1	9	10
	Apr	10	3	7	30
	May	12	6	6	50
	Jun	7	7	1	100
	Jul	8	8	0	100
	Aug	13	13	0	100
	Sep	4	4	0	100
	Oct	10	10	0	100
High	Nov	38	36	2	95
	Dec	28	28	0	100
	Jan	9	0	9	0
	Feb	4	0	4	0
	Mar	12	0	12	0
	Apr	10	8	2	80
	May	14	10	4	71
	Jun	17	17	0	100
	Jul	9	9	0	100
	Aug	18	16	2	90
	Sep	13	13	0	100
	Oct	10	10	0	100

Table 3. Percent occurrence, mean percent number per darter, and mean percent weight of food items in the alimentary canal of 65 *Etheostoma lepidum* collected from the low-flow environment of the Comal River, November 2019-October 2020.

	Percent Occurrence	Mean percent number per fish	Mean % by weight
Coleoptera			
Elmidae	0	0	0
Psephenidae	6.2	1.3	< 0.1
Diptera			
Chironomidae	23	8.3	1.8
Culicidae	0	0	0
Stratiomyidae	0	0	0
Thaumaleidae	0	0	0
Unidentified	0	0	0
Ephemeroptera			
Baetidae	29	11	30
Leptohyphidae	12	3.7	10
Leptophlebiidae	1.5	0.3	1.5
Unidentified	17	5	10
Trichoptera			
Hydropsychidae	0	0	0
Hydroptilidae	20	10	6.1
Philopotamidae	0	0	0
Unidentified	0	0	0
Unidentified insects	15	0.3	3.9
Non-insects			
Amphipoda	60	51	32
Cladocera	15	8.6	< 0.1
Decapoda	1.5	0.3	4
Tricladida	0	0	0
Algae	7.7		< 0.1

Table 4. Percent occurrence, mean percent number per darter, and mean percent weight of food items in the alimentary canal of 68 *Etheostoma lepidum* collected from the high-flow environment of the Comal River, November 2019-October 2020.

	Percent Occurrence	Mean percent number per fish	Mean % by weight
Coleoptera			
Elmidae	14	2.5	< 0.1
Psephenidae	1.5	0.2	< 0.1
Diptera			
Chironomidae	35	17	1.6
Culicidae	1.5	0.2	< 0.1
Stratiomyidae	1.5	0.2	2.4
Thaumaleidae	1.5	0.2	< 0.1
Unidentified	2.9	0.4	1.6
Ephemeroptera			
Baetidae	46	17	47
Leptohyphidae	0	0	0
Leptophlebiidae	4.4	0.5	0.4
Unidentified	10	3.5	4.8
Trichoptera			
Hydropsychidae	47	45	13
Hydroptilidae	26	6.2	8.3
Philopotamidae	1.5	0.2	2.5
Unidentified	10	2.5	1.5
Unidentified insects	32	0.2	4.8
Non-insects			
Amphipoda	24	4.2	10
Cladocera	1.5	0.2	< 0.1
Decapoda	0	0	0
Tricladida	1.5	0.2	0.9
Algae	4.4		0.7

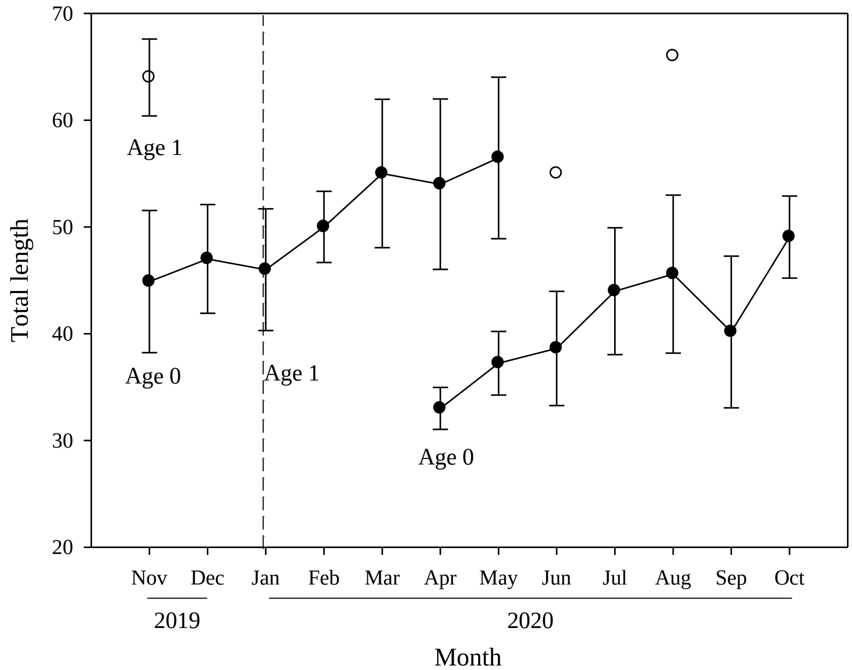

Figure 1. Estimated total lengths (black circles; mean ± 1 SE) for age-0 and age-1 Greenthroat Darters *Etheostoma lepidum* taken monthly from November 2019 to October 2020, calculated from FiSAT 1.2 modal progression analysis. White circles represent total lengths (mean ± 1 SE) taken from ≤ 3 individuals within estimated age-1 fish. Dashed line denotes January 1, when age-0 individuals become age-1 individuals.

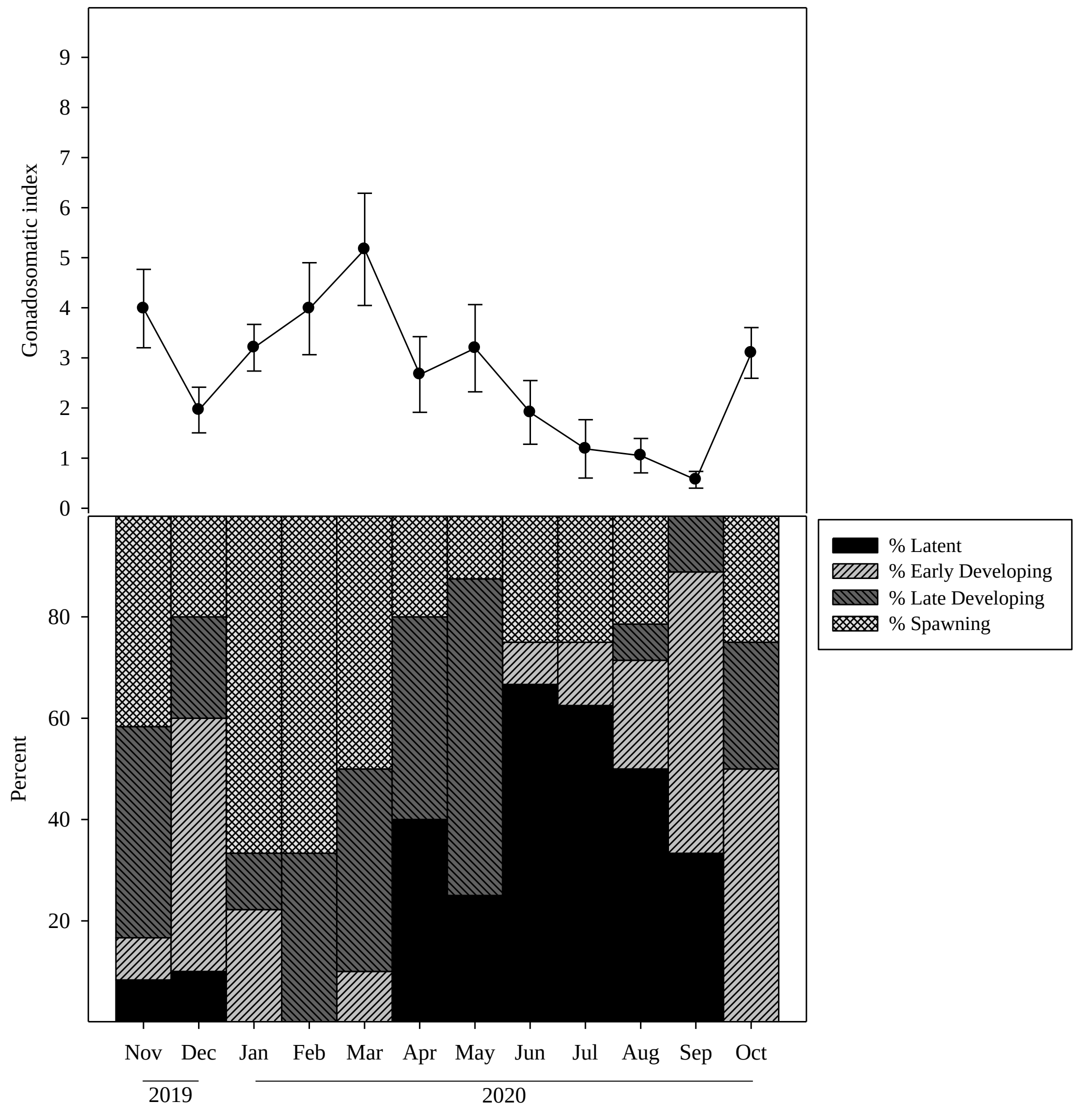

Figure 2. Mean (± 1 SE) monthly gonadosomatic index (GSI) for 119 female Greenthroat Darters *Etheostoma lepidum* taken from low- and high-flow environments from November 2019 through October 2020 (top graph). Percent occurrence of ovarian stages (latent, early developing, late developing, and spawning females) plotted by month (bottom).

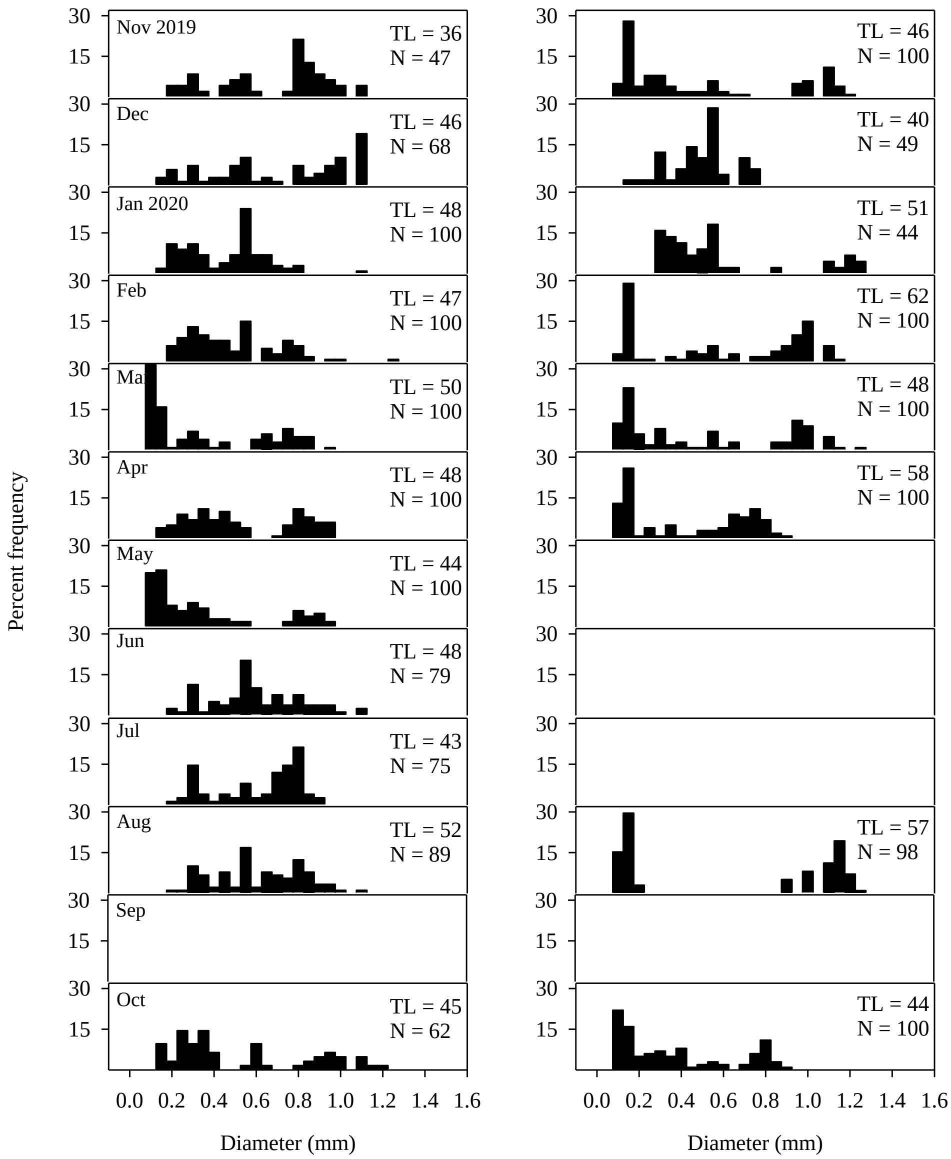

Figure 3. Percent frequency of oocyte size in mature ovaries of Greenthroat Darter *Etheostoma lepidum* taken from the low-flow environment from November 2019 to October 2020 (one female sampled per month). The left panel is the low-flow environment, the right panel is the high-flow environment.

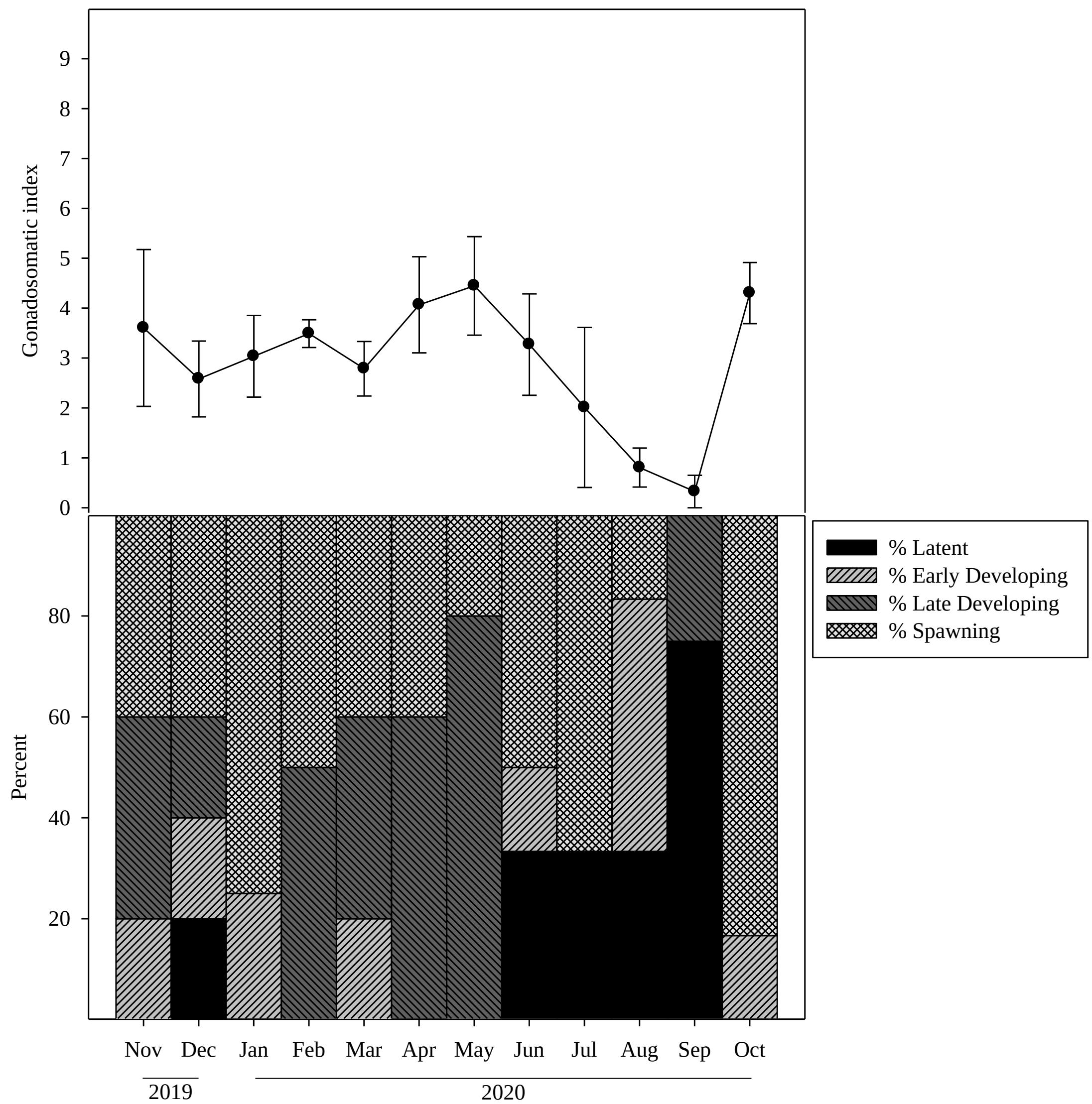

Figure 4. Mean (± 1 SE) monthly gonadosomatic index (GSI) for Greenthroat Darters (*Etheostoma lepidum*) taken from the low-flow environment from November 2019 to October 2020 (top panel); ovarian stages by month for latent, early developing, late developing, and spawning females (bottom panel).

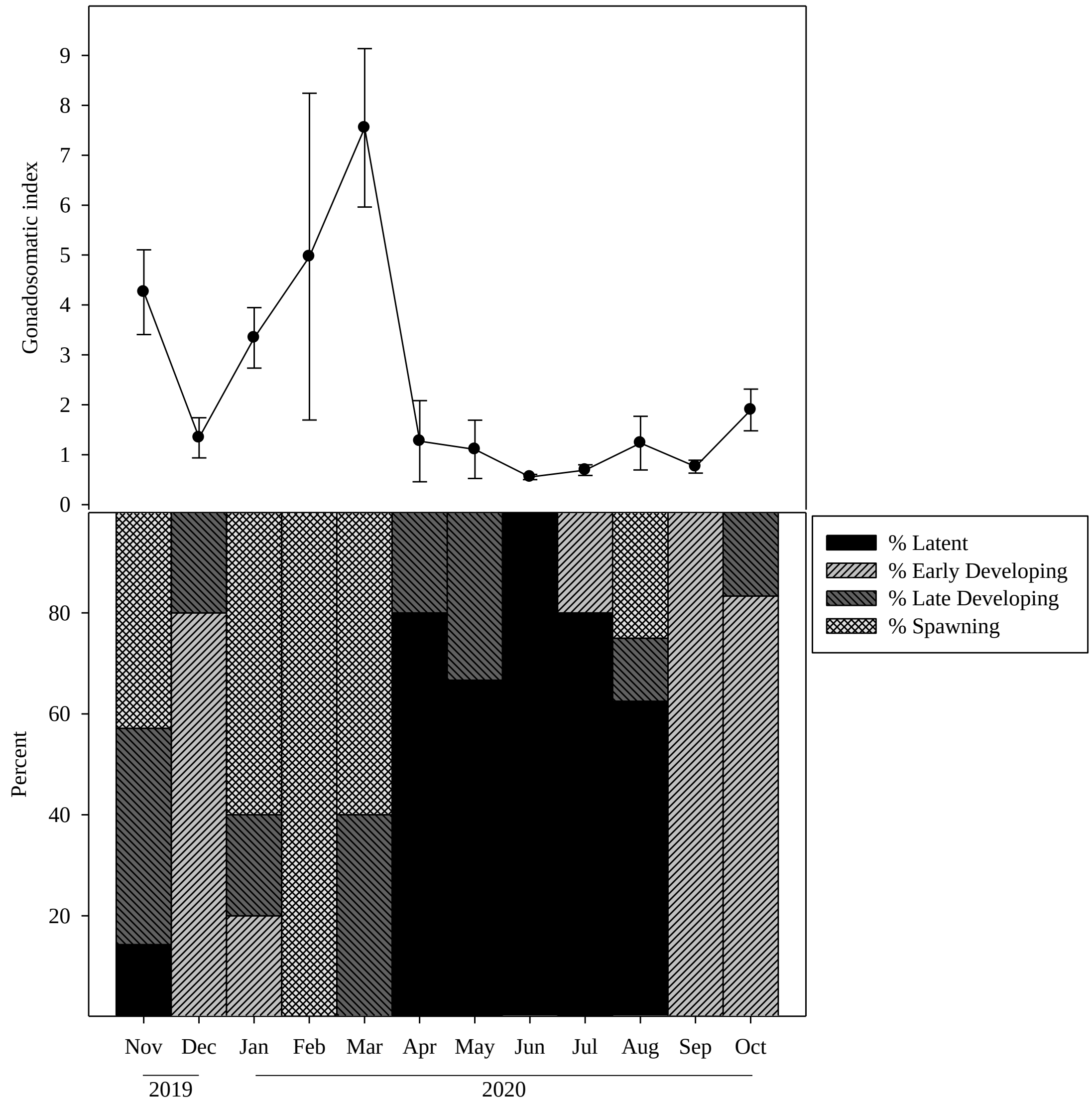

Figure 5. Mean (± 1 SE) monthly gonadosomatic index (GSI) for Greenthroat Darters (*Etheostoma lepidum*) taken from the high-flow environment from November 2019 to October 2020 (top panel); ovarian stages by month for latent, early developing, late developing, and spawning females (bottom panel).

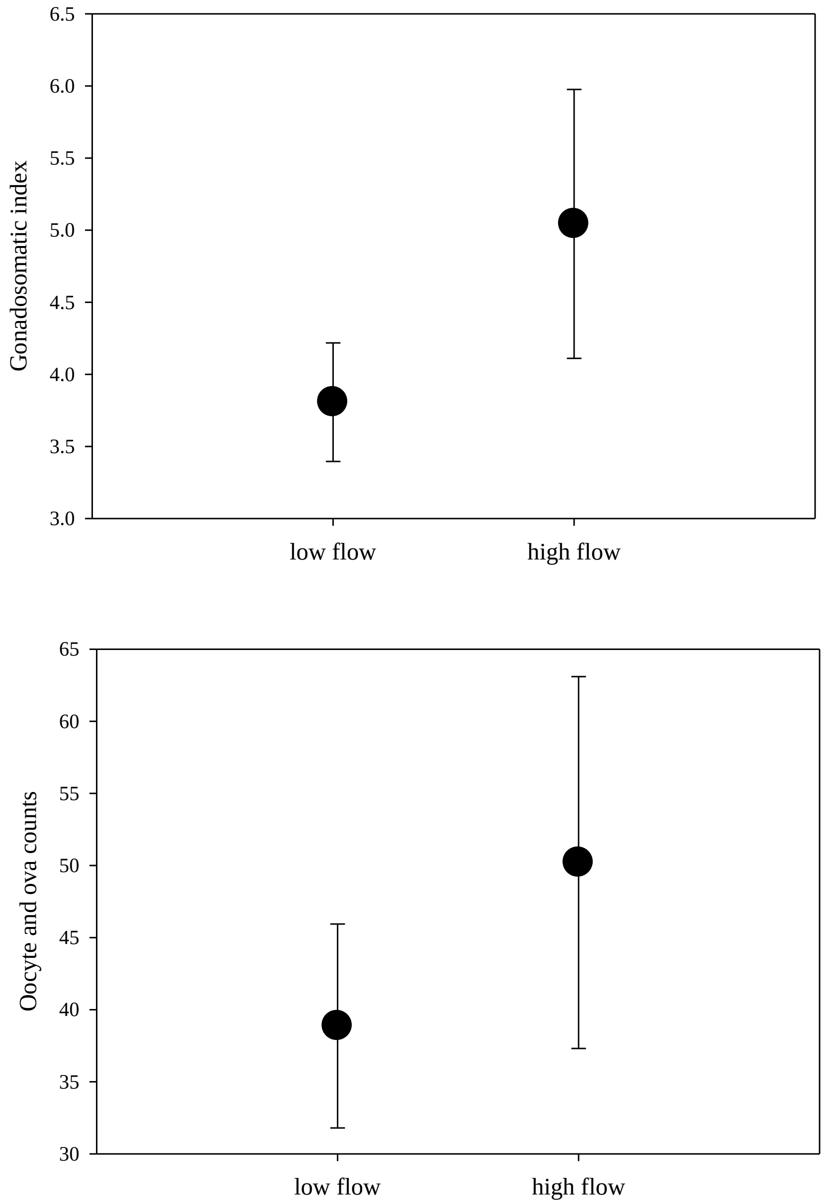

Figure 6. Mean (± 1 SE) gonadosomatic index (GSI) of age-1 female Greenthroat Darters (*Etheostoma lepidum*) at the low- and high-flow environments (top). Mean (± 1 SE) oocyte and ova counts of age-1 spawning female Greenthroat Darters (*Etheostoma lepidum*) at the low- and high-flow environments (bottom).

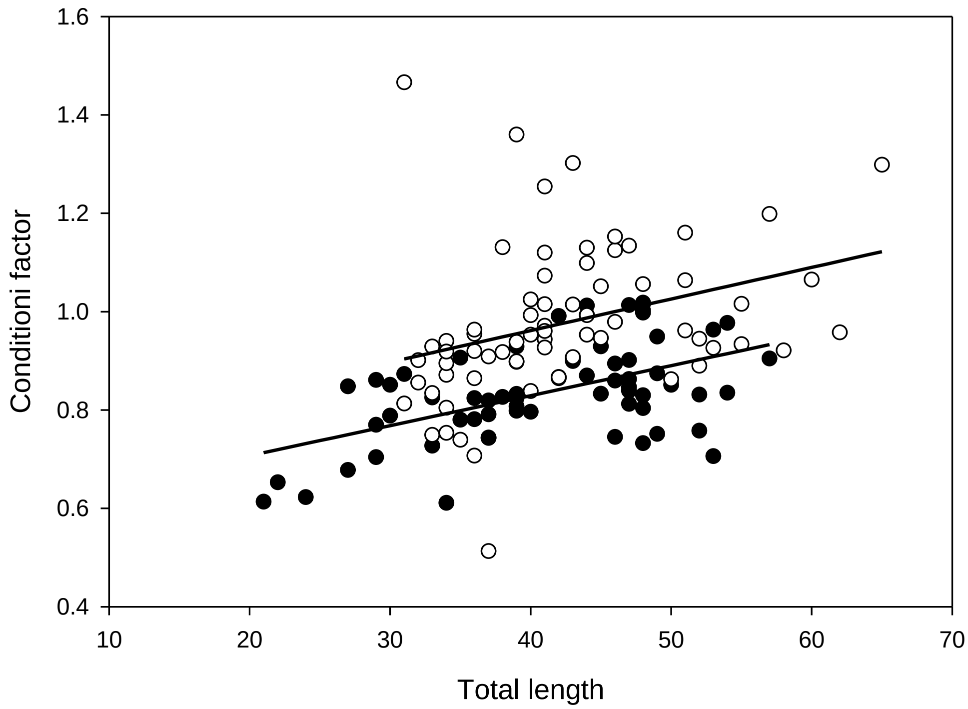

Figure 7. Condition factor per individual Greenthroat Darter (*Etheostoma lepidum*) by environment, the low-flow environment (black circles) and the high-flow environment (white circles) have similar slopes.

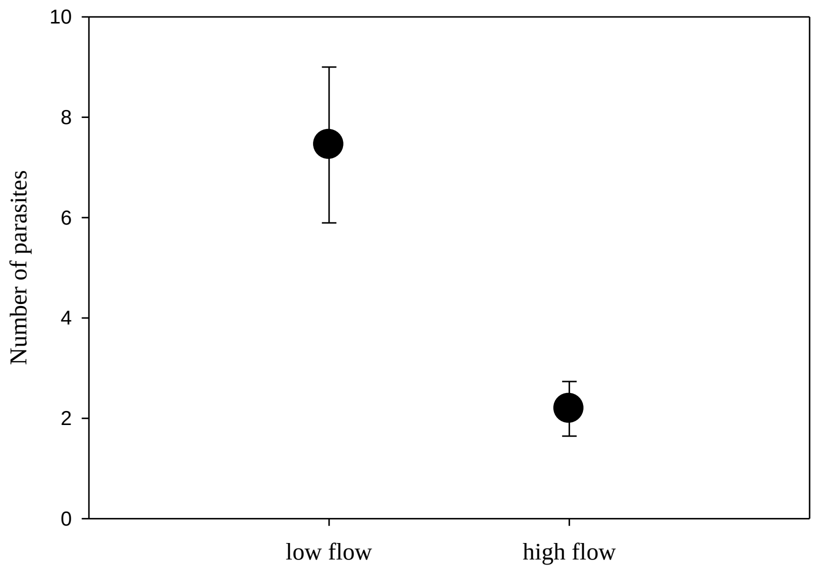

Figure 8. Mean (± 1 SE) number of parasites per Greenthroat Darter (*Etheostoma lepidum*) by site at the low- and high-flow environments.

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