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Assessing Effects of Impounded Water on Life History, Reproduction, and Diets of a Fluvial Specialist Fish

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ABSTRACT.—Species richness and abundances of fluvial specialist fishes often decrease within waters impounded by dams, but mechanisms underlying these decreases are poorly understood. Purpose of this study was to assess the effects of impounded water on fluvial specialist Greenthroat Darter *Etheostoma lepidum* by quantifying differences in life history (*i.e.*, age structure, life span), reproduction (*i.e.*, gonadosomatic index [GSI], stages of ovarian development), and stomach contents (*i.e.*, diet items and parasites) between a population taken from a lentic environment (Lake Site) and a lotic environment (River Site). Among fishes taken from both sites, Greenthroat Darters lived up to 1.5 y, spawned for 11 mo, and consumed primarily aquatic insects and crustaceans. Differences in reproduction were not detected between populations at the Lake Site and at the River Site. Greenthroat Darters taken from Lake Site consumed fewer diet items, fewer aquatic insects, and greater number of crustaceans than those taken from River Site; however, diet weight, percent stomach fullness, and percent empty stomachs were similar between sites. Greenthroat Darters taken from Lake Site exhibited lower condition factors than those taken from River Site, which corresponded with a greater number of parasites (*i.e.*, Acanthocephala and Nematoda) in individuals taken from Lake Site. Differences quantified herein were not sufficient to cause extirpation of Greenthroat Darters in the impounded waters, given the species has persisted in the impounded water at least since the late 1800s; however, differences in diets and parasites might explain the lower abundance of Greenthroat Darters in Lake Site compared to River Site.

INTRODUCTION

The damming of flowing waters typically causes shifts in pre-existing fish communities upstream, within, and downstream of dams (Liew *et al.*, 2016; Turgeon *et al.*, 2019). Within impounded waters, common patterns in fish community shifts include increased richness and abundances of generalist, predatory, and nonnative fishes and decreased richness and abundances of fluvial specialist fishes. Turgeon *et al.* (2019) compiled and summarized processes that contribute to community shifts of fish in impounded waters, including stocking of sportfishes (*e.g.*, increases in predatory fishes, which increases predation on fluvial specialist fishes) and changing lotic environments to lentic environments. Creation of lentic environments is thought to affect negatively fluvial specialist fish behavior and reproduction (*i.e.*, lack of plasticity for lentic environments; Turgeon *et al.,* 2019) leading to their reductions in species richness and abundances in impounded waters; however, specific mechanisms of impounded waters on fluvial specialist fish behavior and reproduction are poorly understood.

The average age of dams within the USA is 57 y (U.S. Army Corps of Engineers, 2018) with most of the dams constructed 50 to 75 y ago (Hansen *et al.*, 2020). Updating aging dam infrastructure will be a necessity (Ho *et al.*, 2017). With about 50% of the 91,000 dams classified as small (*i.e.*, ≤7.6 m in height; U.S. Army Corps of Engineers, 2018) and typically located in low-order streams, a mechanistic understanding of how impounded waters affect headwater fluvial specialist fishes will be beneficial, along with societal services (*e.g.*, water

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storage, flood control) and repair costs, in the planning of updating or removing aging infrastructure. Similar to effects of large dams (>15 m; majority of dams assessed by Turgeon *et al.*, 2019), small dams and resulting impounded waters are associated with shifts in pre-existing fish communities 66% of the time (Hedden *et al.*, 2021) and increases in the number of generalist cyprinids and centrarchids with loss of fluvial specialist fishes (Taylor *et al.*, 2001; Kashiwagi and Miranda, 2009). However, Holcomb *et al.* (2016) reported that fluvial specialist fishes were unaffected by small impoundments, indicating that small dams differentially affect pre-existing fish communities. Understanding how fluvial specialist fishes respond to impounded waters will aid in the understanding of differential effects of dams on pre-existing fish communities and inform the process of updating dam infrastructure.

The purpose of this study was to assess the effects of impounded water on life history aspects, reproduction, and diets of a headwater fluvial specialist fish, Greenthroat Darter *Etheostoma lepidum* (subgenus *Oligocephalus*). Greenthroat Darter is considered a riffle specialist with its largest abundances observed in riffles with vegetation (Hubbs and Strawn, 1957), but is found occasionally in vegetated pools (Kuehne and Barbour, 2015). Greenthroat Darter associates with thermally stable headwater streams of the Edwards Plateau in Texas (Hubbs, 1995; Craig *et al.*, 2016) with a disjunct population located among springs in the Black River in New Mexico (Sublette *et al.*, 1990). Greenthroat Darter is widely distributed within the Edwards Plateau of Texas with abundances ranging from rare to occasional (Faucheux *et al.*, 2019, supplemental data). Spawning season is 8 mo long (Oct.–May; Hubbs, 1985) based on the production of larvae from adults held in a laboratory and 10 to 12 mo based on the presence of ripe females observed in the field (Hubbs and Strawn, 1957). Females produce multiple batches of demersal and adhesive eggs attached to vegetation and sides of aquaria (Hubbs and Strawn, 1957). Otherwise, life history information (*e.g.*, number of age groups, life span), reproduction (*e.g.*, gonadosomatic index [GSI], stages of ovarian development), and diets have not been reported for Greenthroat Darters taken from the wild.

This study consisted of two primary objectives: (1) quantify number of age groups, life span, condition factors, monthly female gonadosomatic index (GSI), ovary and oocyte stages, batch fecundity, and stomach contents (*i.e.*, diet items and parasites) of two Greenthroat Darter populations taken from lentic and lotic environments of a headwater stream within the Edwards Plateau; and (2) to contrast age groups, life span, condition factors, monthly female GSI, ovary and oocyte stages, batch fecundity, and stomach contents of Greenthroat Darters taken from the lentic environment (Lake Site), and those taken from the lotic environment (River Site). The first objective was necessary to infer general patterns in life-history, reproduction, and diet using all available data from both environments. Our predictions for the first objective were that Greenthroat Darter populations will consist of three age groups with a maximum life span of up to 2 y 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 eight months described by Hubbs (1985) given fishes in other thermally stable headwater streams of the Edwards Plateau typically have spawning seasons ranging from 9 to 11 mo (Folb, 2010; Perkin *et al.*, 2012; Robertson *et al.*, 2016), and that diet will consist of aquatic insects, based on the diets reported for the other members of subgenus *Oligocephalus* (Conchos Darter and Mexican Darter; Contreras-Balderas, 1977; Page, 1983). Related to the second objective, an *a priori* prediction was that Greenthroat Darter reproduction would be less (*i.e.*, lower GSI, lower batch fecundity) in Lake Site than in River Site, based on the observation that reductions in flows (*i.e.*, shifts towards more lentic conditions) are reported to decrease reproduction of another spring-associated fish (Fountain Darter *Etheostoma fonticola*) within the Edwards Plateau (Mora *et al.*, 2013). Additionally, as we viewed this study as exploratory, we also compared other quantified variables (*i.e.*, diets, number of parasites, and condition factors) between the two sites in order to detect possible impounded water effects.

METHODS

STUDY AREA

Lake Site and River Site were located within the Comal River (Comal County, Texas; Fig. 1). The Comal River originates from multiple spring outflows of the Edwards Aquifer and flows 6 km before merging with the Guadalupe River (Brune, 1981). Headwaters of the Comal River were impounded by two structures (Landa Falls Dam, 4.0 m in height, and Landa Park Lake Dam, 2.7 m in height), forming Landa Lake in the late 1840s or 1850s (Landa Lake Estates, 1904). Prior to dam construction, water emerged from numerous headwater springs and flowed into the Old Channel. The Mill Race was trenched about 750 m from headwater springs to a bluff overlooking Dry Comal Creek, diverting water to cascade over the bluff to operate a mill. Additional material was added to the bluff to control water outflow, which is now called Landa Falls Dam and marking the upper end of the New Channel with permanent water flow directed into the former channel of Dry Comal Creek. Landa Park Lake Dam was built across the Old Channel to elevate water into the Mill Race.

Lake Site was located in Landa Lake (29.720361, –98.128583), and River Site was located in the New Channel of the Comal River (29.42210, –98.07519), immediately downstream from Landa Falls Dam. Lake Site and River Site are two of 10 biomonitoring survey sites that were sampled twice a year as part of the Edwards Aquifer Habitat Conservation Plan (RECON Environmental, Inc., 2012). From 2014 through 2020, water quality (*i.e.*, water temperature, pH, dissolved oxygen, and specific conductance) were measured at each site twice a year for seven years with a YSI-65 or YSI-85 hand-held meter. A total of 318, 5-m seine hauls or substrate kicks into a seine (3.0×1.8 m common sense seines; mesh size: 3.2 mm) in Lake Site and 114, 5-m seine hauls or substrate kicks into a seine in River Site was made to quantify fish community. After each seine haul or kick, water depth (m) and current velocity (m/s; Marsh McBirney 2000 hand-held velocity meter) were recorded within each 5-m area. Percent substrate and vegetation coverage were visually estimated within each 5-m area. Vegetation type was identified to the lowest practical taxonomic level.

Lake Site was a 2300 m^2 area with a mean flow (± 1 SD) 0.1 (0.09 m^3/s; Nichols, 2015). The site is subjected periodically and briefly to higher flows from precipitation events and surface runoff. Water depths (mean: 1.03 m, SD: 0.39, max: 2.3 m; 2014–2020) and current velocities (mean: 0.05 m/s, SD: 0.59, max: 0.80 m/s) are influenced by the two downstream dams, creating greater depths and slower current velocities than would be expected without the dams. Substrates consisted primarily of gravel (42%), followed by silt (24%), and cobble (16%). Mean vegetation coverage was 46% with vegetation predominantly consisting of filamentous algae (32%), bryophytes (20%), detrital algae (8%), and *Cabomba* (5%). Fish community consisted of 18 species and 3849 individuals. Greenthroat Darter was the eighth most abundant species (5.7% in relative abundance). Mean density (± 1 SD) of Greenthroat Darter was 0.02 (0.06) individuals per m.

River Site was within an 875-m^2 riffle with a mean daily flow (± 1 SD) of 2.6 (2.09) m^3/s (2014–2020; USGS Station 08168932). The riffle is the largest riffle habitat within the Comal River and has the largest concentration of Greenthroat Darters among the 10 biomonitoring sites. Mean water depth ($\pm$ SD) was 0.73 m (± 0.28; max: 1.37; 2014–2020). Mean current

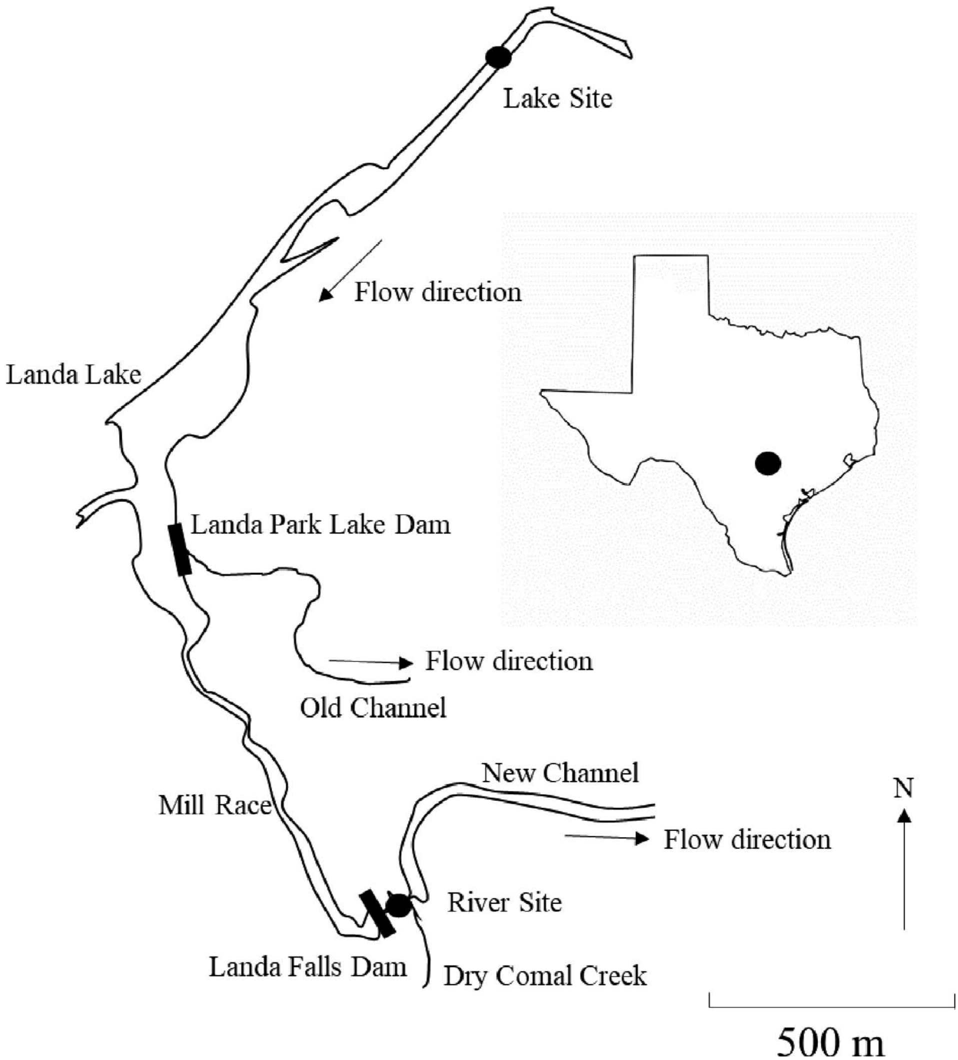

FIG. 1.—Headwaters of the Comal River in New Braunfels (Comal County) Texas. Lake Site was located in the upper end of Landa Lake. River Site was located immediately downstream from Landa Falls Dam

velocity (±1 SD) was 0.40 m/s (±0.25; max: 1.10). Substrates consisted primarily of gravel (49%), followed by cobble (20%), and sand (13%). Mean vegetation coverage was 36% with vegetation predominantly consisting of *Vallisneria* (16%), *Justicia* (14%), and *Ludwigia* (10%). Fish community consisted of 17 species and 870 individuals. Greenthroat Darter was the third most abundant species (11% in relative abundance). Mean density (±1 SD) of Greenthroat Darter is 0.05 (0.18) individuals per m.

Because both sites were within close proximity to spring outflows (Groeger *et al.*, 1997), mean and variability of water quality variables were similar between sites. Mean water temperature (±1 SD) was 23.6 C (0.61) at Lake Site and 23.3 C (0.80) at River Site, mean dissolved oxygen was 7.4 mg/L (2.23) at Lake Site and 8.4 mg/L (0.88) at River Site, and mean specific conductance was 570 µS/cm (12) at Lake Site and 534 µS/cm (137) at River Site.

FIELD COLLECTIONS AND LABORATORY PROCESSING

Fish were collected monthly from the two sites for twelve consecutive months (Nov. 2019–Oct. 2020). For each collection and site, a seine net (3 m × 1.8 m; 3.2 mm mesh size) was used to collect fish. Total length (TL), to the nearest mm, was measured on all Greenthroat Darters collected. Up to eight 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 2 wk, rinsed, and then transferred to 70% ethanol. Fish handling and collection were performed in accordance with conditions stipulated by Texas State University IACUC (Protocol 7359) and Texas Parks and Wildlife Department Scientific Permit Number SPR-0601-159.

In the laboratory Greenthroat Darters were removed from 70% ethanol, blotted dry, measured (TL), and weighed to the nearest mg. An incision was made in the abdomen region from the urogenital opening to the 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 diet analysis. Ovaries were separated from the viscera. Ovaries were examined under light microscopy. Ovarian stage and oocyte to ova maturation were classified using modified descriptions specific to darters (Heins and Baker, 1989; Heins *et al.*, 1992; Heins *et al.*, 1995). 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 and weighed to the nearest mg. Only spawning ovaries, when available, were processed for oocyte and ovum diameters and for batch fecundity. Measurement of oocyte and ovum diameters were taken from one of the ovaries, if both ovaries were about the same size, or from both ovaries if one ovary was about twice the size of the other, which was found in 10 of the 36 females with spawning ovaries. For each female, the spawning 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 micrometer on a microscope was calibrated with a stage micrometer measuring 1 mm, for the first 100 oocytes and ova in field of view, oocyte and ova diameters were measured to the nearest 0.01 mm. The number of ova, recognizable by the ovum indentation and oil droplet (Nichols, 2015), were enumerated to estimate batch fecundity. The number of 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. A longitudinal incision was made along the stomach to expose ingested food items. Stomach fullness, the percent amount of the stomach filled by food items, was estimated visually and independently by two observers from 0 to 100% in increments of 10% (Childs *et al.*, 1998). The two estimates were then averaged and recorded for each individual fish. All food items were removed from the stomach, blotted with cloth to remove excess moisture, and weighed to the nearest mg. Food items were separated and identified to the lowest level of confident identification, which was family-level of classification for insects and order-level of classification for other arthropods. Unidentified insects were grouped into their own category, and unidentified families of insects were grouped by order. Food items were enumerated and weighed by classification level. Food items for each fish were expressed as total number of food items, total weight of food items, percent number (the number of a classification level divided by total number of food items multiplied by 100), and percent weight (the weight of a classification level divided by the total weight of food items multiplied by 100). Algae were noted as occurring, weighed, and expressed as percent weight. Parasites from within the stomach and viscera were removed, identified to genus-level of classification, enumerated, and weighed.

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 and sexes 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 Nov. 2019 and Oct. 2020. Gonadosomatic index (GSI: [mass of ovary/ mass of eviscerated fish] times 100) was calculated for each female. Mean GSI was calculated by site and month as well as the combined sites by month. Months with elevated GSIs (*i.e.*, >2%) 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 were indicative of spawning season (*i.e.*, occurrence of ovum). Gonadosomatic indices among months for all females and for females with late developing and spawning ovaries and numbers of ova among females with spawning ovaries were assessed between sites with one-factor ANOVAs. Length frequency histograms were constructed for oocyte and ova diameters to determine if single or multiple clutch production during the reproductive season (Heins and Baker, 1989).

Percent occurrence, mean percent number, and mean percent weight of food items were calculated for all fish, fish from Lake Site, and fish from River Site. Percent occurrence was calculated as the percentage of Greenthroat Darters consuming a diet category (*i.e.*, family or order-level of classification or algae). Mean percent number was calculated as the mean of individual percent number for each diet category, and mean percent weight was calculated as the mean of individual percent weight for each category (Bowen, 1996). Total number of food items, total weight of food items, and parasite counts were compared between sites with one-factor ANOVAs ($\alpha = 0.05$). Condition factor (weight/TL^3 multiplied by 100) was calculated for each fish as a measure of fitness and compared between sites with a one-factor ANOVA.

RESULTS

Total lengths were measured from 308 male and female Greenthroat Darters captured from the two sites. 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 (Fig. 2). Estimated life span was approximately 1.5 y. First detection of age-0 fish in 2020 was in Apr.

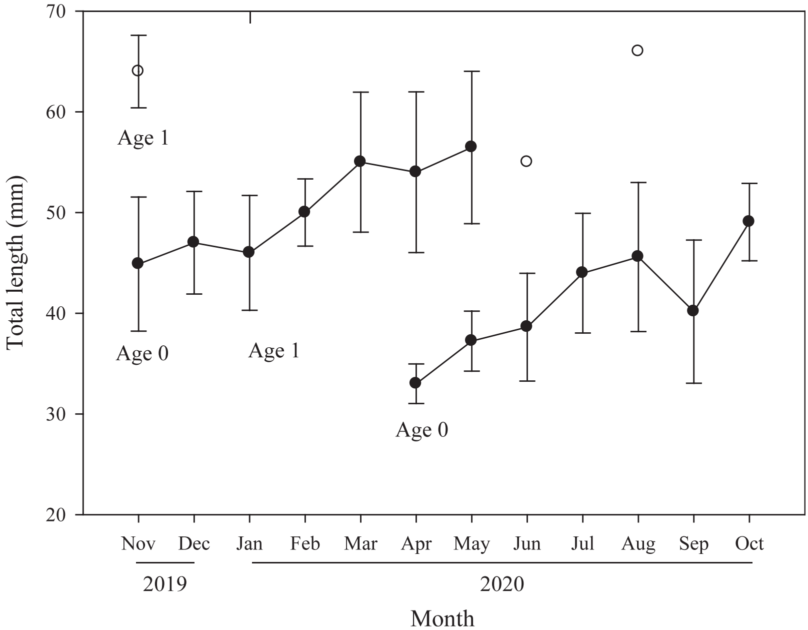

FIG. 2.—Estimated total lengths (black circles; mean ± 1 SE) for age-0 and age-1 Greenthroat Darters *Etheostoma lepidum* taken monthly from Nov. 2019 to Oct. 2020, calculated from FiSAT 1.2 modal progression analysis. White circles represent total lengths (mean ± 1 SE) taken from ≤3 and excluded in the modal progression analysis. Dashed line denotes Jan. 1, when age-0 individuals become age-1 individuals

Among the 308 Greenthroat Darters captured, 119 female Greenthroat Darters and 14 males Greenthroat Darters were taken for laboratory processing. Among females, GSIs were elevated from Nov. 2019 through May 2020 and again in Oct. 2020 (Fig. 3). Latent ovarian stage was observed in Nov. and Dec. and from Apr. to Sep. Early developing ovarian stage was observed in all months, except Feb., Apr., and May. Late developing ovarian stage was observed in all months, except Jun. and Jul. Spawning ovarian stage was observed in all months except Sep. 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, multiple modes were observed in diameter-frequency distributions (Fig. 4).

Food items were quantified in 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 fullness of food items was 63% ± 2.8. At both sites, Greenthroat Darters consumed 20 identifiable diet categories. Amphipoda was the highest in percent occurrence (41%), followed by Baetidae (37%), and Chironomidae (29%) (Table 1). Hydropsychidae was the highest in mean percent number (29%), followed by Amphipoda (20%), Baetidae (15%), and Chironomidae (14%). Baetidae was the highest in mean percent weight (38%), followed by Amphipoda (21%).

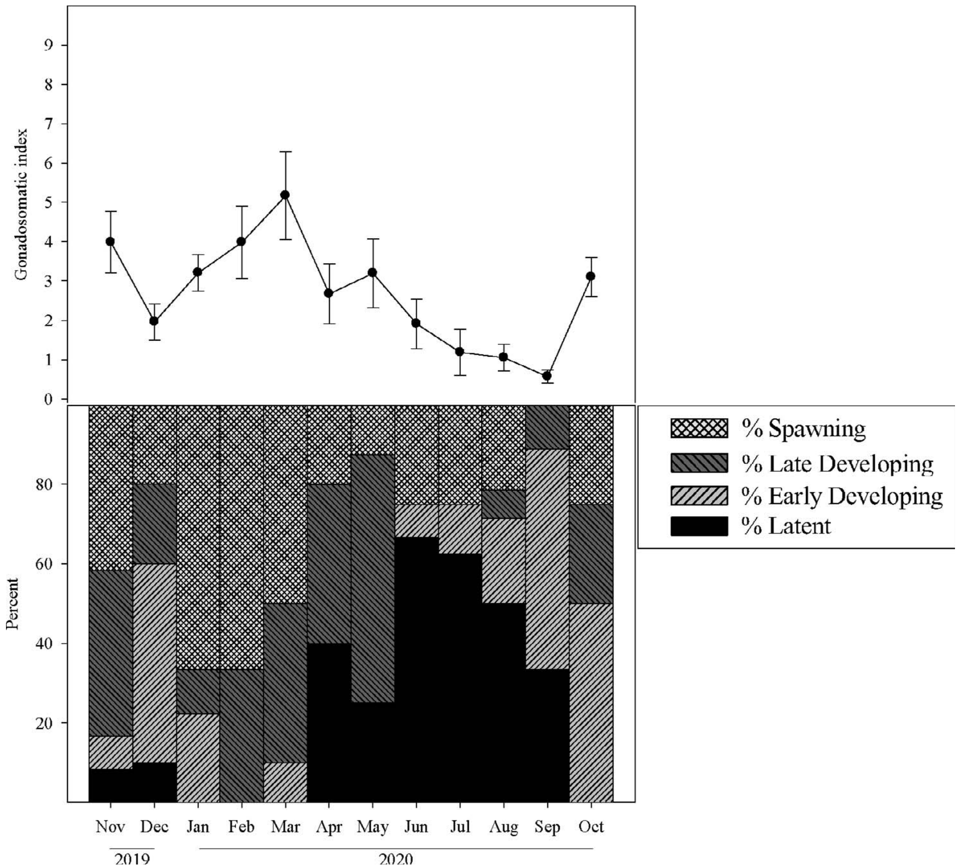

FIG. 3.—Mean (± 1 SE) monthly gonadosomatic index (GSI) for 119 female Greenthroat Darters *Etheostoma lepidum* taken from Lake Site and River Site from Nov. 2019 through Oct. 2020 (top panel). Percent occurrence of ovarian stages (latent, early developing, late developing, and spawning females) plotted by month (bottom panel)

COMPARISONS BETWEEN LAKE SITE AND RIVER SITE

Among the 308 male and female fish captured, 126 Greenthroat Darters (range: 10–60 mm TL) were captured from Lake Site, and 182 Greenthroat Darters (range: 29–67 mm TL) were captured from River Site. Age-0 fish composed 54% of the population in Lake Site and 81% of the population in River Site across all months (Table 2). Female GSI was not different ($F_{1,117} = 2.4$; $P = 0.13$) between individuals taken from Lake Site (mean GSI: 3.0; 1 SE: 0.28; N: 58) and River Site (mean GSI: 2.3; 1 SE: 0.33; N: 61), although GSIs from females taken from Lake Site remained elevated for a longer period than those from River Site (Fig. 5). However, River Site had fewer age-1 fish (9%) than Lake Site (age-1 fish: 22%). Excluding age-0 fish from the calculations because most had not obtained sexual maturity, GSIs of age-1 females with late developing and spawning ovaries were not different ($F_{1,29} = 1.8$; $P = 0.19$) between Lake Site (mean GSI 3.8; 1 SE: 0.41; N: 18) and River Site (mean GSI 5.0; 1 SE: 0.93; N: 13). Among age-0 and age-1 fishes with spawning ovaries, batch fecundity

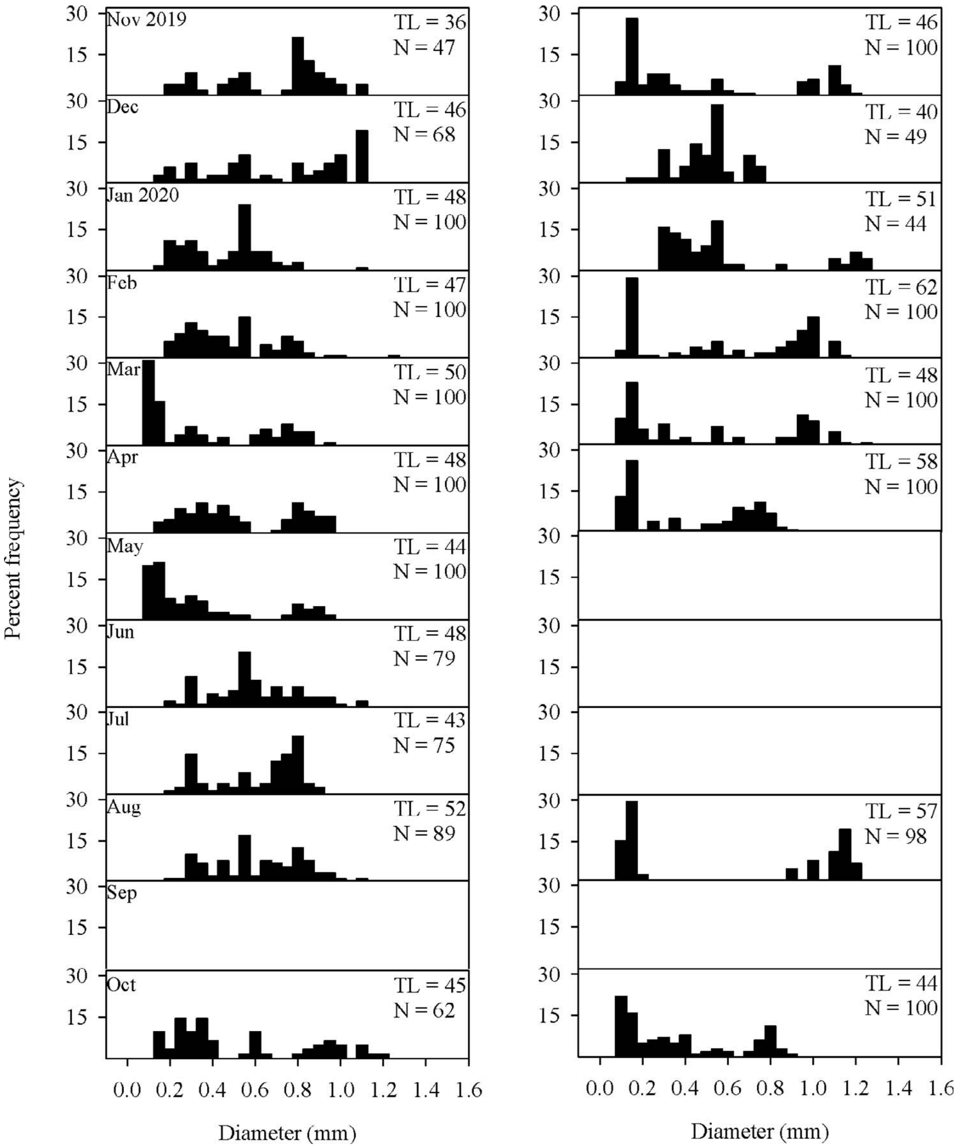

FIG. 4.—Percent frequency of oocyte size in spawning ovaries of Greenthroat Darter *Etheostoma lepidum* (one female sampled per month) taken from the Lake Site (left panel) and River Site (right panel) from Nov. 2019 to Oct. 2020. Blanks indicate the absence of spawning ovaries in a month

was not different ($F_{1,34} = 0.32$; $P = 0.58$) among females taken from Lake Site (mean batch fecundity: 29.3; 1 SE: 5.1; N: 23) and in River Site (mean batch fecundity: 34.5; 1 SE: 8.6; N: 13).

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 Lake Site, and 68 fish (range:

TABLE 1.—Percent occurrence, mean percent number, and mean percent weight of diet categories in the stomachs of 133 Greenthroat Darters *Etheostoma lepidum* collected from two sites on the Comal River, Nov. 2019 through Oct. 2020

	Percent occurrence	Mean percent number	Mean percent 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

31 to 65 mm TL) were taken from River Site. Percentages of empty stomachs were 7.7% in fish taken from Lake Site and 8.8% in fish taken from River Site. Among fish with at least one food item in their stomach, mean (±1 SE) percent stomach fullness was 66% ± 3.7 in fish taken from Lake Site and 59% ± 4.2 in fish taken from River Site. Number of food items per individual differed ($F_{1,131} = 4.9$; $P = 0.03$) between individuals taken from Lake Site (mean number of food items consumed per individual: 4.6 items; 1 SE: 0.54; N: 65) and those taken from River Site (mean: 8.35; 1 SE: 1.54; N: 68). However, weight of food items was not different ($F_{1,131} = 0.01$; $P = 0.93$) between fish taken from Lake Site (mean weight of food items consumed per individual: 2.00 mg; 1 SE: 0.26; N: 65) and those taken from River Site (mean: 1.96 mg; 1 SE: 0.31; N: 68). Among individuals taken from Lake Site, Amphipoda was the highest in percent occurrence (60%), mean percent number (51%), and mean percent weight (32%; Table 3). Among individuals taken from River Site, Hydropsychidae was the highest in percent occurrence (47%) and mean percent by number (45%) with Baetidae being the highest in mean percent weight (47%).

Greenthroat Darters taken from Lake Site often appeared to be more emaciated than those taken from River Site. The relationship between condition factor and TL differed (ANCOVA; interaction term; $F_{1,132} = 6.6$; $P = 0.01$) between fish taken from Lake Site and fish taken from River Site. Mean (±1 SE) condition factors across all lengths of 0.83 (±0.12)

TABLE 2.—Total number of Greenthroat Darters *Etheostoma lepidum* collected from the Lake Site and the River Site by month, separated by their estimated ages and taken from the Comal River, Nov. 2019 through Oct. 2020

	Month	N	Percent of age-0 fish	Percent of age-1 fish
Lake	Nov.	12	100	0
	Dec.	5	100	0
	Jan.	25	0	100
	Feb.	10	0	100
	Mar.	10	10	90
	Apr.	10	30	70
	May	12	50	50
	Jun.	7	86	14
	Jul.	8	100	0
	Aug.	13	100	0
	Sep.	4	100	0
	Oct.	10	100	0
	Total	126	54	46
River	Nov.	38	95	5
	Dec.	28	100	0
	Jan.	9	0	100
	Feb.	4	0	100
	Mar.	12	0	100
	Apr.	10	80	20
	May	14	71	29
	Jun.	17	100	0
	Jul.	9	100	0
	Aug.	18	89	11
	Sep.	13	100	0
	Oct.	10	100	0
	Total	182	81	19

for individuals taken from Lake Site and 0.98 ($\pm$0.02) for individuals taken from River Site (Fig. 6). In addition, numbers of endoparasites were greater ($F_{1,131} = 10.5$; $P < 0.01$) in individuals taken from Lake Site (mean count: 7.4; SE: 1.55) than those taken River Site (mean count: 2.2; SE: 0.55). Across both sites, 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.

DISCUSSION

Study results supported our initial predictions on the Greenthroat Darter spawning season being longer (*i.e.*, 11 mo) than the 8 mo described from laboratory observations (Hubbs and Strawn, 1957) and Greenthroat Darter diets consisting largely of aquatic insects, although a large proportion of their diets also included amphipods and cladocerans in individuals taken from Lake Site. However, some of the initial predictions were not supported. The number of age groups (*i.e.*, two) and longevity (*i.e.*, 1.5 y) was less than those reported for other members of *Oligocephalus* (Edwards, 1997). Addtionally, lower reproductive effort (*i.e.*, lower GSI, lower batch fecundity) was not detected in comparisons between individuals at

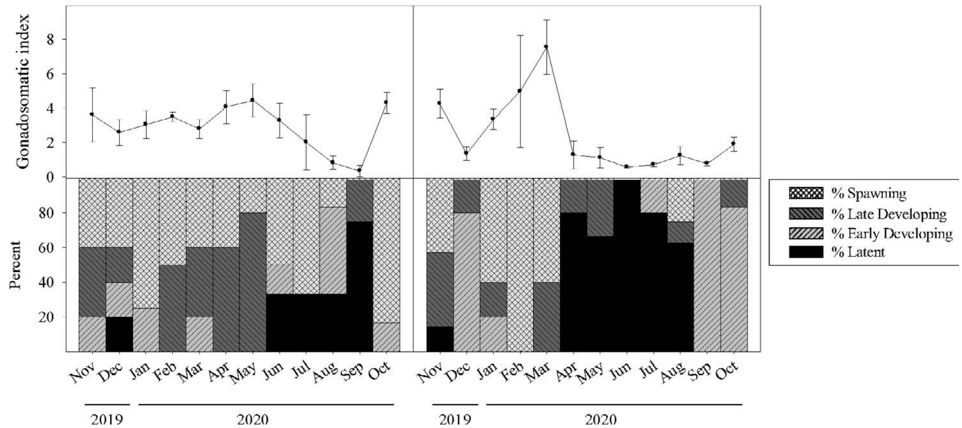

FIG. 5.—Mean (± 1 SE) monthly gonadosomatic index (GSI) for female Greenthroat Darters *Etheostoma lepidum* taken from the Lake Site (N: 58; top left panel) and River Site (N: 61, top right) from Nov. 2019 to Oct. 2020. Percentage of ovarian stages by month for latent, early developing, late developing, and spawning females at the Lake Site (bottom right) and River Site (bottom left)

the Lake Site and River Site as initially predicted. Differences among individuals between sites, however, were observed for diets, parasite load, and condition.

In this study, Greenthroat Darters invested energy into reproduction all year with spawning during an 11 mo period (Oct.–Aug.) based on the presence of ova. The 11 mo spawning period reported herein is longer than the 8 mo previously reported by Hubbs (1985), although Hubbs and Strawn (1957) suggested a longer spawning period (10–12 mo, although no evidence of spawning in Jul.) based on the presence of ripe females (expressed by stripping; Strawn and Hubbs, 1956). Members of the genus *Etheostoma* typically spawn during the Spring and Summer for about 3 to 4 mo (Page, 1983). However, an often-reported pattern among a phylogenetically diverse group of fishes associated with thermally stable water temperatures is a 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 mo period, excluding Sep. (Nichols, 2015). Guadalupe Darter *Percina apristis*, taken from the thermally stable waters of the San Marcos River, spawns for 11 months, excluding Jul. (Folb, 2010). In addition, within the thermally stable waters of the San Marcos River, Ironcolor Shiner *Notropis chalybaeus* spawns for 10 mo (*i.e.*, Mar. through Dec.), which is 4 mo longer than conspecifics from nonthermally stable waters (Perkin *et al.*, 2012). Extending spawning season length in thermally stable waters could represent 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 waters 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 a 1 or 2 mo 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).

TABLE 3.—Percent (%) occurrence, mean % number per darter, and mean % weight per darter of diet categories in the stomachs of 65 Greenthroat Darters *Etheostoma lepidum* collected from the Lake Site and 68 Greenthroat Darters from the River Site, Nov. 2019 through Oct. 2020

	Percent occurrence		Mean percent number		Mean percent by weight	
Categories	Lake Site	River Site	Lake Site	River Site	Lake Site	River Site
Coleoptera						
Elmidae		14		2.5		<0.1
Psephenidae	6.2	1.5	1.3	0.2	<0.1	<0.1
Diptera						
Chironomidae	23	35	8.3	17	1.8	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	29	46	11	17	30	47
Leptohyphidae	12	0	3.7		10	
Leptophlebiidae	1.5	4.4	0.3	0.5	1.5	0.4
Unidentified	17	10	5	3.5	10	4.8
Trichoptera						
Hydropsychidae		47		45		13
Hydroptilidae	20	26	10	6.2	6.1	8.3
Philopotamidae		1.5		0.2		2.5
Unidentified		10		2.5		1.5
Unidentified insects	15	32	0.3	0.2	3.9	4.8
Non-insects						
Amphipoda	60	24	51	4.2	32	10
Cladocera	15	1.5	8.6	0.2	<0.1	<0.1
Decapoda	1.5	0	0.3		4	
Tricladida		1.5		0.2		0.9
Algae	7.7	4.4			<0.1	0.7

Failure to detect differences in reproductive effort among individuals between Lake Site and River Site was surprising. Mora *et al.* (2013), based on a modeling exercise, reported that a transition from more lotic to more lentic environments (*i.e.*, reductions in spring flow) would decrease recruitment of age-0 Fountain Darters. Fountain Darter is a slackwater species (Behen, 2013); therefore, we expected a riffle specialist, like Greenthroat Darter, would be more susceptible to reductions in flow with the creation of more lentic conditions (Aadland, 1993). Other riffle specialists, such as Orangethroat Darter *Etheostoma spectabile* (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 turn increases survival of the individuals. However, Greenthroat Darters in Lake Site do have access to swifter current velocities and higher flows (*i.e.*, more voluminous spring outflows within Landa Lake than those near Lake Site), which are about 3 km downstream from Lake Site. Why Greenthroat Darters remain in the more lentic environment of Lake Site is unknown.

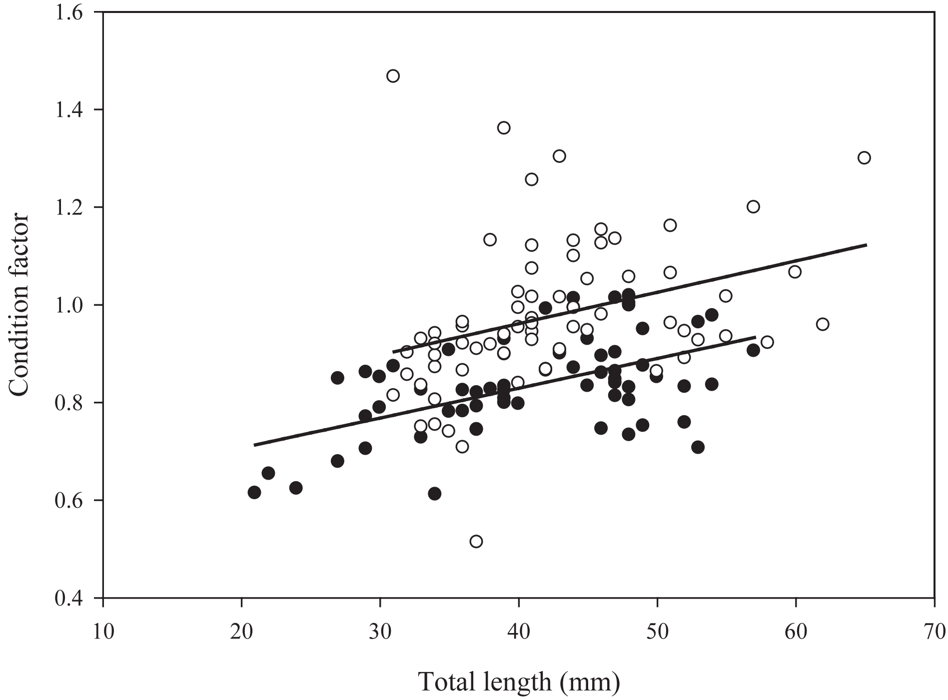

FIG. 6.—Condition factor per individual Greenthroat Darter *Etheostoma lepidum* by environment, the Lake Site (black circles) and the River Site (white circles) have similar slopes

Greenthroat Darters consumed fewer diet categories, fewer Hydropsychidae and Baetidae, and greater percent number of Amphipoda in Lake Site than in River Site. Food item weight, percent stomach fullness, and percent of empty stomachs were similar. Differences in diets were attributed largely to the association of prey items with lotic or lentic environments. Amphipoda tends to be more abundant in pools than riffles (Dahl and Greenberg, 1996) and 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 to fast flows or with rapid flows, and all nine of the species of *Baetis* were associated with moderate to fast flows.

Greater number of parasites in Greenthroat Darters taken from Lake Site was attributed to diet differences between sites and the associations of food items for either lotic or lentic environments. The intermediate host of *Leptorhynchoide* is Amphipoda, which was consumed in greater frequency, number, and weight in Lake Site than in River Site. Prevalence of parasites is positively related to degradation of aquatic systems (Artim *et al.*, 2020), potentially like that found at the Lake Site in this study due to impoundment, and inversely related to current velocity (Hallett and Bartholomew, 2008). 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, such as the endoparasite *Leptorhynchoide* (Phylum Acanthocephala), can be independent of levels of habitat degradation (Artim *et al.*, 2020). Nevertheless, we tentatively attributed the emaciated appearance and low condition factors of Greenthroat Darters in Lake Site to the high prevalence of the endoparasites, given that food item weight, percent empty stomachs, and percent stomach fullness were similar between fish in Lake Site compared to River Site. Alternatively, the emaciated appearance in Lake Site could be related to phenotypic variation (*i.e.*, shallower body) associated with low flow and more lentic environments (Franssen *et al.*, 2013), although shallower body (*e.g.*, more streamlined body) is typically associated with lotic environments (Langerhans, 2008). Additionally, the emaciated appearance could be attributed to another selection pressure as synthesized by Franssen *et al.* (2013), such as predator density effects on fishes with more elongated bodies found in fishes (*i.e.*, Poeciliidae) with predators than in predator-free populations (Langerhans *et al.*, 2004). Based on biomonitoring surveys taken between 2014 and 2020, relative abundance of predatory fishes (*i.e.*, Largemouth Bass *Micropterus salmoides*, Green Sunfish *Lepomis cyanellus*, and Warmouth *L. gulosus*; Goldstein and Simon, 1999) was 25% in Lake Site, whereas relative abundance of predatory fishes in River Site was 3%.

Although differences in diets, parasite load, and condition were observed among fish between Lake Site and River Site, these differences were not sufficient to cause localized extirpation of a fluvial specialist fish in the impounded water. Greenthroat Darter has persisted in Landa Lake at least since their first report in Landa Lake in 1891 (Hendrickson and Cohen, 2015), including an extreme low-flow period in the 1950s that is largely to blame for the reported extirpation of the Fountain Darter by the 1970s (Schenck and Whiteside, 1976). However, differences in diets, parasite load, and condition might be sufficient, along with greater abundances of predatory fishes (Marsh-Matthews *et al.*, 2011) at Lake Site, to explain the lower relative abundances of Greenthroat Darter in Lake Site (5.5%) than in River Site (10%) observed during biomonitoring surveys. Thus, this study might have captured some of the specific mechanisms that could explain decreased richness and abundances of fluvial specialist fishes with the creation of impounded waters. More studies are needed from a larger number of fluvial specialist fishes and impounded waters of various sizes to fully explore and identify the mechanisms of impoundment effects on pre- existing fish communities and the conditions that lead to fish community shifts or not (*i.e.*, differential effects).

One potential limitation in understanding differential effects lies with the term "fluvial specialist fish." Kinsolving and Bain (1993) defined fluvial specialist fishes as those that were "almost always reported from streams and rivers and were often described as requiring flowing-water habitats throughout life." Using this definition, not all fluvial specialist fishes have the same traits with respect to mobility, migration, feeding, reproduction, size, and shape (Lima *et al.*, 2016) and therefore can be differentially affected by impounded water. As suggested by Turgeon *et al.* (2019), expanding the traits of the fluvial specialist fish (*e.g.*, Greenthroat Darter: sedentary, benthic habitat use, invertivore, phytolithophil, no parental care, batch spawner) could provide greater insight into how specific traits among fluvial specialist fishes are or are not susceptible to extirpation and reduced abundances in impounded waters.

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