SPRING FLOW AND HABITAT-MEDIATED EFFECTS
ON REPRODUCTIVE EFFORT OF
THE FOUNTAIN DARTER

by

Harlan Thomas Nichols, B.S.

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Committee Members:

Timothy Bonner, Chair
Kenneth Ostrand
Joseph Veech
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DEDICATION

This work is dedicated to my parents who always took the time to foster my curiosity with the natural world.
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ABSTRACT

Reductions in Edwards Aquifer spring flows are hypothesized to reduce reproductive effort of spring-associated fishes. Purposes of this study were to test relationships among spring flow, associated habitat changes, and reproductive effort of the federally-listed Fountain Darter *Etheostoma fonticola*, a spring-associated fish inhabiting Comal and San Marcos rivers of central Texas. Study objectives were to quantify annual reproductive effort (i.e., ovarian stages, gonadosomatic index, and batch fecundity) across a gradient of flows (0.01 m³/s to 3.7 m³/s) and aquatic habitats using natural and anthropogenically-altered stream reaches (N = 4) within the Comal and San Marcos rivers to represent in situ flow reductions. Contrary to previous studies reporting constant year-round spawning effort, annual reproductive cycle of the Fountain Darter consisted of an optimal reproductive season (January through April), and tailing reproductive season (May through August), lack of spawning in September, and a leading reproductive season (October through December). Among reproductive seasons, stages of ovarian condition, gonadosomatic indices, and batch fecundity generally were not different along a flow gradient or among habitats, though two exceptions were noted. Gonadosomatic index and batch fecundity were greater ($P < 0.05$) at the higher flow environment (4.1 m³/s) during optimum reproductive season and greater ($P < 0.05$) at the lowest flow environment (0.01 m³/s) during the leading reproductive season. Collectively, seasonality of reproductive effort was similar to sister taxa (*Cypress Darter* E. proeliiare, Least Darter *E. microperca*), though protracted, and
reproductive effort was not related consistently to flow environment observed during the study period. However, other measures of reproductive effort (e.g., numbers of larvae hatched, survival of larvae through recruitment age) are necessary to quantify in order to assess the relationship among Fountain Darter population viability and flow environments.
I. SPRING FLOW, HABITAT, AND REPRODUCTION

Introduction

Groundwater extraction and subsequent reductions in surface water flows are threats to endemic aquatic fauna (Van der Kamp 1995, Acreman et al. 2000, Kollaus et al. 2015). Within the karst Edwards Plateau region of Texas, numerous springs ranging from seeps (< 0.01 m$^3$/s) to very large spring complexes (> 9 m$^3$/s) emanate from Trinity-Edward aquifers (Brune 1981). Sustained groundwater pumping reduces spring flows (Van der Kamp 1995). Historical record (1975-1995) of average annual pumping in the Balcones Fault Zone of the Edwards Aquifer was greater than the current groundwater extraction limit set by the Edwards Aquifer Authority (Loáiciga et al. 2000). Demand for groundwater is expected to increase as the region shifts towards a more arid climate (Loáiciga et al. 2000). Effects of groundwater extraction and surface water flow reductions are a greater ecological concern during periods of below average precipitation, specifically if surface water flows are insufficient to maintain aquatic habitats within springs inhabited by endemic species (Crowe and Sharp 1997). Spring outflows and corresponding spring runs of streams within the Edwards Plateau, collectively referred to as spring complexes, are considered as evolutionary refugia (Craig et al. 2015) and support many endemic aquatic species (Bowles and Arsuffi 1993). Two of the largest spring complexes within the Edwards Plateau region are the Comal River (8.5 m$^3$/s; USGS Station # 0816900) and upper San Marcos River (5.0 m$^3$/s; 08170500). Upper San Marcos River and Comal River are characterized by exceptional water quality (Groeger et al. 1997, Fahlquist and Slattery 1997) and intact fish communities despite being encompassed within urbanized watersheds (Kollaus et al. 2015).
Concerns with groundwater extraction and federally-listed threatened and endangered species resulted in policies that restrict groundwater withdrawals in the Edwards Aquifer (Votteler 1998). The Edwards Aquifer Authority was established as an agency responsible for setting critical minimum limits of spring flows, and hence maximum allowable groundwater extraction, to protected species viability (McCarl et al. 1999). Restricting groundwater withdrawal for municipal and agricultural purposes has economic consequences. Current pumping limits are derived from Senate Bill 1477 (Texas Legislature 1993) and restrict pumping to 49,000 hectare-meters per year, which places an estimated $1.1 million loss collectively to businesses, agricultural practices, and other water users dependent upon groundwater (McCarl et al. 1999). Groundwater and surface water uses are increasing as urban and rural development continues to increase (Fitzhugh and Richter 2004). Species protected under the Endangered Species Act exist within Comal River and upper San Marcos River; this requires mediation by agencies like the Edwards Aquifer Authority to strike a balance between the conflict of water requirements for municipalities, industry, and agriculture and the designated water that is essential to maintain critical habitats of those species.

The Fountain Darter, *Etheostoma fonticola*, is a federally-listed endangered species, located in Comal River and upper San Marcos River (Bonner and McDonald 2005). The species is often referred to as ‘the little fish that roared’ due to litigation concerning groundwater rights and the persistence of surface water integral for species viability (Votteler 1998). Concern exists for population persistence during periods of low flows attributed to groundwater withdrawal, given that the Comal River experienced reduced spring flows (0.15 m³/s) in 1956 (Cox et al. 2009) and that the Fountain Darter
was considered extirpated in following years (Schenck and Whiteside 1976). As such, current conservation efforts for the Fountain Darter include water quantity management as the Comal River and upper San Marcos River are linked by essentially the same water supply (Ogden et al. 1986). Regulation of groundwater withdrawals depends on surface flows and its effects on species (USFWS 1996, Saunders 2001). Management of endangered species, and subsequent habitat, is guided by a habitat conservation plan (www.eahcp.org). A flow-based reproductive assessment is listed as priority applied research and has not been completed for the Fountain Darter. Information would fill gaps in current knowledge of biological responses to reductions in water quantity and quality that could affect populations.

Information about Fountain Darter response to reductions in base flows is currently obtained by ecological modeling. System-wide flow reductions leading to a complete dewatering event are unpredictable and uncommon (Humphries and Baldwin 2003), and, therefore, difficult to capture and document. Sustained decreases in spring flows leading to extremely low flow conditions are rare in the Comal River (Cox et al. 2009) and non-existent historically for upper San Marcos River (Kollaus et al. 2015). As such, modeling provides a framework to predict the level of population persistence during such events. Current model predictions indicate that Fountain Darter populations remain stable at, or above, 2.8 m³/s, and that population levels are not severely affected until spring flow reaches 1.7 m³/s or below (Mora et al. 2013). Three processes, which are assumed to be affected by reductions in spring flow and associated changes in abiotic conditions, are recruitment, development, and mortality (Mora et al. 2013). Recruitment, specifically reproductive aspects of recruitment such as ovarian development leading to
clutch production, is a process with predictable habitat-level abiotic effects related to spring flow, including seasonality (Schenck and Whiteside 1977), temperature (Brandt et al. 1993, McDonald et al. 2006), and suitability of spawning habitat (Phillips et al. 2011). An assessment of reproductive processes within low-flow conditions for the Fountain Darter has not been conducted.

Objectives of this study were to describe the annual reproductive cycle (i.e., ovarian stages, Gonadosomatic index, batch fecundity) of Fountain Darters across a gradient of flow environments and to assess the relationship between reproductive effort (i.e., GSI and batch fecundity) and flow environments, specifically flow rates and physicochemical parameters. Variation in flow environments was obtained by sampling four reaches of the Comal River and upper San Marcos River with different flow rates (0.10 ± 0.09 m³/s to 3.7 ± 0.60 m³/s) related to natural and anthropogenic variation in stream channels. Predictions were 1) Fountain Darters will spawn year-round (Schenck and Whiteside 1977) unless water temperatures exceed optimum conditions (Brandt et al. 1993, McDonald et al. 2006) or suitable spawning habitats (i.e., aquatic vegetation and filamentous algae) are lacking (Phillips et al. 2011), 2) reproductive effort will be reduced when flow is between 4.6 to 8.5 m³/s (Schenck and Whiteside 1977), and 3) reproductive effort will be reduced when flow is < 2.8 m³/s (Mora et al. 2013).
Methods

Study areas were three sites on the Comal River (Comal County, Texas) and one site on the upper San Marcos River (Hays County, Texas). Both the Comal River and San Marcos River were sampled to include the extent of the range of the Fountain Darter. Flows are modified by several low-head dams in each river, creating a heterogeneous mix of river channels with slack to swift water habitats, silt to boulder substrates, and non-vegetated to densely-vegetated areas. Sites were selected to represent a flow environment gradient, ranging from low to high flows, in areas considered as critical habitat for the Fountain Darter (USFWS 1996; Figure 1). Site 1 was located in upper spring run of the Comal River (mean flow JAN – DEC, 2014 ± 1 SD: 0.1 ± 0.09 m³/s, range in monthly mean flow: < 0.01 – 0.28 m³/s, source: BIOWEST, unpublished data). Flows were estimated monthly at Site 1 using an averaged velocity and depth cross-sectional method while flows at all other sites were obtained from USGS gauging stations. Site 2 was located in Old Channel of the Comal River (1.4 ± 0.2 m³/s, 1.0 – 1.9 m³/s, USGS Station 08168913). Flow at Site 2 is regulated by a head gate to maintain year around flows at 1.4 m³/s and was sampled because it has a relatively stable flow rate compared to the other sites. Site 3 was located in New Channel of the Comal River (2.0 ± 0.95 m³/s, 0.34 – 3.60 m³/s, USGS Station 08168932). Site 4 was located in City Park of the San Marcos River (3.67 ± 0.60 m³/s, 2.94 – 6.52 m³/s, USGS Station 08170500).

At each site, sexually-mature female Fountain Darters (> 24 mm in total length; Brandt et al. 1993) were collected monthly from January through December 2014 under authorization from Federal Fish and Wildlife Permit TE236730-1, Texas Parks and Wildlife SPR-0601-159, and Texas State University IACUC Protocol 0116-0218-02. All
males and sexually immature females (< 24 mm TL) were promptly released to the immediate area of capture. A total of five females were targeted for harvest at each site from habitats with no vegetation (i.e., sand, gravel, and cobble substrates), short-growing vegetated habitats (i.e., vegetation height < 50% of water depth), and tall-growing vegetated habitats (i.e., vegetation height > 50% of water depth). Stratified sampling of habitats was conducted to ensure sampling of all habitats given possible influences of habitat on Fountain Darter reproduction (Phillips et al. 2011). Moving from a downstream to upstream direction at each site, Fountain Darters were taken with fine-meshed dipnets or short (< 5 m) seine hauls. From each habitat, harvested females were anesthetized in a lethal concentration of tricaine methanesulfonate and preserved in a 10% buffered formalin solution. In close proximity to the area of harvest (< m²), water depth (m) and current velocity (m/s) were measured using a Marsh-McBirney Flo-mate portable electromagnetic flow meter and a 1.2 m Rickly USGS top setting wading rod within vegetation and above (where applicable), and at 60% of the water depth for habitats without vegetation. Water properties were measured using a YSI 556 Multi-parameter System and included dissolved oxygen (mg/l), pH, temperature (°C), and specific conductance (µS/cm). Percent detritus and percent substrate composition were visually estimated. Percent substrate was assigned using a modified Wentworth scale (silt: <0.06 mm, sand: 0.06–1.99 mm, gravel: 2–63 mm, cobble: 64–255 mm, boulder: >256 mm, and bedrock).

In the laboratory, female Fountain Darters remained in 10% formalin for two weeks, were washed with water, and transferred to 70% ethanol solution. Total length (mm) was measured and ovaries were excised and cleaned of any residual tissue. Ovaries
and eviscerated bodies were patted dry and weighed on a Mettler Toledo AB54-S Analytical balance. Gonadosomatic index (GSI) was estimated as the ratio of whole ovary weight to eviscerated body weight (liver, intestinal tract, and other viscera removed). Eviscerated weight was used as a method to control for variability in gut fullness and liver size.

Ovarian stage and oocyte to ova maturation were classified using modified descriptions specific to darters (Heins and Baker 1989, Heins et al. 1992, Heins 1995). A small subsample of ovaries were selected and used for histological examination to confirm classifications of oocyte and ovarian stages (Appendix A). Ovarian stages and oocyte to ova maturation were classified visually into four categories (Appendix B). Latent ovaries contained only pre-vitellogenic oocytes, early developing ovaries were slightly larger than latent ovaries and contained vitellogenic oocytes that appeared opaque and only moderately enlarged, late developing ovaries were larger and contained oocytes that were greatly enlarged and opaque, cream, or amber in color with and without chorions distinctly visible, and spawning ovaries contained ripe ova characteristic of ‘class I ova’ described by Schenck and Whiteside (1997) and elaborated upon by Burr and Ellinger (1980). Late developing ovaries containing ripening oocytes (same as mature ripening ovaries described by Heins 1995) were selected to standardize GSI values for testing among flow environments as the use of ovaries containing ripening oocytes constrains variability in weight to a developmental stage that is nearing the end of vitellogenesis and prior to ovulation (Heins and Baker 1993, Heins 1995). Ripening oocytes were identified by wider chorions, cream to amber in color, and greatly enlarged without the indentation characteristic of ova (Appendix C). Counts of ripening oocytes...
were made in the left ovary and doubled to estimate batch fecundity. Counts were made in both lobes if the whole ovary was obviously asymmetrical. Ovaries that contained just a few ripening oocytes (< 3) were excluded from analysis of batch fecundity due to the possibility of including ovaries in a clutch estimate that were in the process of oocyte reclamation (Heins 1995).

Statistical analyses were used to assess habitat differences among sites and months and reproductive effort among sites (a surrogate for flow environments) and through time. Habitats occupied by Fountain Darters were quantified with principal component analysis (PCA: Proc PRINCOMP, SAS 9.3, SAS Institute Inc., Cary, North Carolina). Vegetation type (i.e., no vegetation, short-growing vegetation, tall-growing vegetation) were coded as categorical variables (i.e., 0 or 1). Continuous variables (i.e., water temperature, pH, dissolved oxygen, water depth, current velocity and specific conductance) and percentage variables (i.e., silt, sand, gravel, cobble, boulder, and detritus) were z-score transformed before being used in the PCA analysis (Krebs 1999). Differences in habitats among sites were assessed with a one-factor ANOVA (α = 0.05) and a post-hoc Fisher’s Least Significance Difference applied to the PC score along PC axes 1 and 2 for each habitat occupied by a Fountain Darter. Each PC score for PC axes 1 and 2 was grouped among sites (treatment effect) independent of month. Differences in habitats among months were also assessed with a separate one-factor ANOVA (α = 0.05) and a post-hoc Fisher’s Least Significance Difference wherein each PC score was grouped among months (treatment effect) independent of site. For reproductive effort, GSI of all ovaries and proportion of ovarian stages were assessed across months to describe annual reproductive cycle. Monthly differences in GSI of all ovaries were tested
with a one-factor ANOVA ($\alpha = 0.05$) and a post-hoc Fisher’s Least Significance Difference. Monthly GSIs were correlated with monthly PC 1 and 2 scores by site and month to assess the influence of site and monthly habitat differences in GSI. For the assessment of GSI containing ripening oocytes and batch fecundity related to flow environments, an unexpected seasonality in GSIs was detected. To control for the effect of season, tests of GSI with ripening oocytes and batch fecundity by flow environment (i.e., site) were constrained by reproductive season (defined in Results). Per reproductive season, GSI with ripening oocytes and batch fecundity among flow environment were individually assessed with a one-factor ANOVA ($\alpha = 0.05$), followed by post-hoc Fisher’s Least Significant Difference test.
Results

Fountain Darters were taken from habitats generally characterized by moderately shallow water with sluggish current velocities in predominantly silt, gravel, or cobble substrates (Table 1). Principal component (PC) axes 1 and 2 explained 32% of the total variation in habitat parameters taken at the location of each harvested Fountain Darter (Figure 2). Principal axis 1 explained 18% of total variation and described a substrate and vegetation gradient with strong negative loadings associated with silt substrate and strong positive loadings associated with areas of little to no macrophyte coverage (bare substrates), gravel and sand substrates with greater amounts of leafy detritus. Principal axis 2 explained 14% of total variation and described a current velocity, substrate, and water temperature gradient with strong negative loadings associated with warmer water temperatures and gravel substrates and strong positive loadings associated with swifter current velocities and sand substrates. Habitats differed among sites according to PC 1 ($F_{3,528}= 63.94; P < 0.01$) and PC 2 ($F_{3,527}= 179.8; P < 0.01$). Habitats in site 1 consisted of more bare, gravel substrates and warmer water temperatures, indicated by PC axes 1 and 2, respectively. Habitats in Site 4 consisted of swifter current velocities described by PC 2. Habitats in Site 2 and 3 were not distinguishable based on either PC 1 or PC 2. Habitats differed among months for PC 1 ($F_{11,519} = 3.4; P < 0.01$) and PC 2 ($F_{11,519} = 15.2; P < 0.01$). Habitats among months varied more within PC 2 than PC 1, describing a cyclic water temperature gradient from cooler winter temperatures in January to warmer summer temperatures in August, and a return to cooler winter temperatures in December.

Overall reproductive effort, as measured by GSI of all ovaries and ovarian stages, differed through time (Figure 3). Gonadosomatic indices differed among months
with individual values ranging from 0.01 to 22.5 for females between 24 and 42 mm in total length. Mean monthly GSIs (± 1 SE) were greatest from January to April with the greatest mean monthly GSI observed in March (7.8 ± 0.56). Likewise, monthly percentages of females with late developing and spawning ovaries differed among months. Collectively, percentages of late developing or spawning ovaries comprised 80% to 91% of females sampled from January to April, decreased to 25% in August transitioning into September where no spawning ovaries were observed, and increased to 75% in December. Conversely, latent and early developing ovaries increased from May through September. Gonadosomatic indices were not correlated with habitats by site for PC 1 ($r_{df} = 2 = 0.39; P > 0.05$) and PC 2 ($r_{df} = 2 = 0.39; P > 0.05$) and with month for PC 1 ($r_{df} = 10 = -0.02; P > 0.05$) and PC 2 ($r_{df} = 10 = 0.01; P > 0.05$).

Temporal trends in reproductive efforts (i.e., GSI and ovarian stages) were similar across sites (Figure 4). Site-level exceptions were the lack of a GSI increase during June in Site 2, and overall mean GSIs (± 1 SE) were greater in San Marcos River (4.3 ± 0.4) than the three Comal River sites (3.9 ± 0.2). Among sites, number of ripening oocytes (i.e., batch fecundity) per female ranged from 4 to 70. Mean (± 1 SE) number of ripening oocytes per female was greatest during March (28 ± 3) and least from August to October (12 ± 2). Total mean (± 1 SE) number of ripening oocytes per female was 19 (± 0.8). With evidence of seasonal reproductive trends, subsequent assessments of Fountain Darter reproductive effort related to habitat and flow were constrained by season: January through April (optimum spawning season), May through August (tailing spawning season), and September through December (leading spawning season).
Gonadosomatic index of females containing ripening oocytes and batch fecundity of ripening oocytes differed among sites (i.e., flow environments) but not consistently across seasons. Within the Optimum Season, GSI differed \((F_{3,75} = 6.8; \, P < 0.01)\) and batch fecundity differed \((F_{3,75} = 17.5; \, P < 0.01)\) among sites. Gonadosomatic index and batch fecundity were greater at Site 4, the highest flow environment, during the optimum season, than the other three sites (Figure 5). Within the Tailing Season, GSI and batch fecundity did not differ \((P > 0.05)\) among sites. Within the Leading season, GSI differed \((F_{3,53} = 5.6; \, P < 0.01)\) and batch fecundity differed \((F_{3,53} = 5.1; \, P < 0.01)\) among sites. GSI was greater within Site 1 than the other three sites. Batch fecundity at Site 1 did not differ from batch fecundity at Site 4 but was greater than batch fecundity at Sites 2 and 3.
Discussion

Initial predictions were not supported. Spawning was not consistent throughout the year, as previously reported by Schenck and Whiteside (1977). Apparent cessation of spawning in September was not attributed to flow or habitat conditions. Average monthly flows ranged between 0.01 m³/s (Site 1) to 3.1 m³/s (Site 4) in September, which was similar to flows in August (0.01 m³/s at Site 1, 3.3 m³/s at Site 4) and October (0.03 m³/s at Site 1, 3.0 m³/s at Site 4). Habitat conditions were not different (PCA analysis) in September compared to habitat conditions in August and October. Prediction that reproductive effort would decrease at flows between 4.6 to 8.5 m³/s (Schenck and Whiteside 1977) was not supported. For example, maximum daily mean flow at Site 4 was 6.3 m³/s in May 2014 which corresponded with an increase in June 2014 GSI. Prediction that reproductive effort would decrease at flows < 2.83 m³/s (Mora et al. 2013) was not supported. Fountain Darter reproductive effort did not differ among flow environments ranging from 0.17 to 3.1 m³/s during the optimum season among Sites 1 - 3, although GSI was greater at Site 4 with greater flow (mean daily flows, January – April: 4.1 m³/s). Greater GSIs could be related to greater flows at Site 4, but inter-population variation in oocyte production is documented for *Etheostoma* and other percid genera across a variety of habitats for reasons not clearly understood (Marsh 1986, Kellog et al. 1997).

Fountain Darter spawning and reproductive investment was greatest during late Winter and early Spring (January – April; optimum reproductive season), declined late Spring through Summer (May – August; tailing reproductive season) with spawning ceasing in September, and increased during Fall through early Winter (October –
December; leading reproductive season). Reproductive investment, or gonadal preparedness, was year round based on occurrences of early developing and late developing ovarian conditions. Spawning and reproductive investment were largely independent of flow rates observed during the study period. However, increases in GSIs at sites 1, 3, and 4 lagged by a few weeks after a high flow pulse in May, attributed to an area-wide precipitation event, whereas Site 2 did not experience a high flow pulse or increases in GSIs because of flow regulation. Increases in reproductive effort in fish species correspond to high flow pulses within eurythermal streams, although it is unknown if flow pulses are a proximate cue for egg release (Durham and Wilde 2006, Zeug and Winemiller 2007). Spawning and reproductive investment were also largely independent of site-level habitat conditions observed during the study period. Most notably, Site 1 was dominated by gravel and cobble substrates and small amounts of vegetation. Vegetation is considered crucial for Fountain Darter spawning in the wild (Phillips et al. 2011). However, vegetation is often not utilized in hatchery rearing of Fountain Darters as attachment of adhesive eggs to a variety of substrates (e.g. aquaria glass, flow-through pipes, and PVC tubing) is common (Brandt et al. 1993). Numbers of Fountain Darters based on qualitative estimates, numbers of spawning individuals, and reproductive investment of all females suggest lack of vegetation was not a limiting factor. Fishes utilizing an unguarded phytolithophilic spawning strategy exhibit a broader range of suitable spawning habitats with selection more likely based upon availability rather than suitability (Balon 1975). Water temperatures, another habitat parameter often associated with Fountain Darter distribution and abundances, did not exceed upper critical thermal tolerances for survival (34.8 °C, Brandt et al. 1993) or reproduction (>
29°C; Bonner et al. 1998, McDonald et al. 2006). As such, not all reported limiting factors associated with Fountain Darter reproduction were assessed in this study.

Fishes associated with stenothermal systems, like karst spring complexes, generally have protracted spawning seasons (Hubbs 1985). Freshwater fish spawning seasons, in general, are linked to both ultimate and proximate cues, most notably photoperiod for gonadal recrudescence and water temperature for gonadal senescence (Vlaming 1972, Pankhurst and Porter 2003). As such, lengths of spawning season are predictable along a latitudinal gradient (Gotelli and Pyron 1991), though seasonality might differ based on phylogeny (e.g., percids spawning during spring and cyprinids spawn in summer). In stenothermal systems, photoperiod likely remains a cue for gonadal recrudescence but lack of temperature fluctuations, primarily cooler water temperatures for cyprinids and warmer temperatures for percids, do not trigger gonadal senescence (Hubbs 1985, Folb 2010, Perkin et al. 2012); hence, protracted spawning seasons are reported for spring-associated fishes. In the San Marcos River, Ironcolor shiner Notropis chalybaeus, a glacial relict restricted to the upper San Marcos River, has a 10-month spawning season from March through December, whereas conspecifics have spawning seasons ranging from two months (i.e., June and July) in Michigan to six months (i.e., April through September) in Florida (see Perkin et al. 2012 for comparative chart). Likewise, Guadalupe Darter Percina apristis, taken from stenothermal waters of the lower San Marcos River, have a nine month spawning season (i.e., October through June), whereas congenera River Darter Percina shumardi, taken from eurythermal waters of mainstem Guadalupe River, have a five month spawning season (i.e., December through April) (Folb 2010). Of the two sister taxon to the Fountain Darter, the Cypress
Darter *Etheostoma proeliae*, is reported to spawn for a two to four month period from January through April in eurythermal streams depending upon latitude (Burr and Page 1978) and populations of Least Darter *Etheostoma microperca* occurring in stenothermal spring complexes have protracted spawning seasons ranging from February to June, or longer (Burr and Page 1979).

Using multiple spring reaches with different magnitudes of flow to investigate reproductive effort as a surrogate for low flow effects is a novel approach in assessing biotic responses to low or high flow conditions (Craig et al. 2015). Manipulating a spring complex to induce low flow conditions *in situ* is logistically difficult and inadvisable in endangered species habitat. However, the approach used in this study had some limitations. Reducing flows at Site 4 (3.68 m$^3$/s) to Site-1 levels (0.1 m$^3$/s) gradually or abruptly within a spawning season likely will create a different aquatic environment (e.g., issues with dissolved oxygen; greater densities of predators and competitors that could stress the Fountain Darters) than in Site 1, which is a fairly constant low-flow environment. Also, Site 1 water depth was influenced by a downstream dam. Thus, periods of < 0.1 m$^3$/s (June through December) still contained water depths > 0.5 m, which likely would not occur at Site 4 without a downstream dam. Another limitation of this study is that clutch production and spawning is only one part of the recruitment cycle and does not necessarily indicate successful maintenance of populations (Durham and Wilde 2006). As such, predictions concerning reproduction of the Fountain Darter under low flow conditions in this study are, and should be considered, as strictly pertaining to the clutch production cycle observed for one year. Survival of eggs, larvae, and juveniles into adulthood were not taken into account and should be assessed across a flow gradient.
to accurately estimate the sum of effects created by low flows on Fountain Darter recruitment.

This study is part of the validation process for maintaining sufficient populations of the Fountain Darter in the Comal River and upper San Marcos River under low flow conditions. Recovery of endangered species is the goal of any conservation effort. This study directly assessed potential effects of low flows to Fountain Darter reproduction and is part of the process of validation of ecological models that are developed, in conjunction with long-term monitoring, to assess the viability of species within native habitat under different flow conditions. However, ensuring the flow needs of aquatic biota, including the Fountain Darter, will continue to require a balance to be struck between water needs for society and those for critical habitats of aquatic species.
**TABLE 1.**—Habitat characteristics for female Fountain Darters within study sites for the entire period of sampling, parenthesis denote standard error associated with mean.

<table>
<thead>
<tr>
<th></th>
<th>Site 1</th>
<th>Site 2</th>
<th>Site 3</th>
<th>Site 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flow (m$^3$/s)</td>
<td>0.1 ( - )</td>
<td>1.4 (0.01)</td>
<td>2.0 (0.05)</td>
<td>3.7 (0.03)</td>
</tr>
<tr>
<td>Current Velocity (m/s)</td>
<td>0.01 (0.01)</td>
<td>0.08 (0.01)</td>
<td>0.05 (0.01)</td>
<td>0.09 (0.01)</td>
</tr>
<tr>
<td>Depth (m)</td>
<td>0.67 (0.02)</td>
<td>0.71 (0.02)</td>
<td>0.71 (0.02)</td>
<td>0.68 (0.03)</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>23.9 (0.2)</td>
<td>22.5 (0.1)</td>
<td>22.5 (0.1)</td>
<td>21.2 (0.1)</td>
</tr>
<tr>
<td>Minimum</td>
<td>19.9</td>
<td>20.3</td>
<td>18.8</td>
<td>18.6</td>
</tr>
<tr>
<td>Maximum</td>
<td>29.6</td>
<td>24.3</td>
<td>24.9</td>
<td>22.7</td>
</tr>
<tr>
<td>Dissolved Oxygen (mg/l)</td>
<td>8.1 (0.2)</td>
<td>8.2 (0.1)</td>
<td>8.4 (0.1)</td>
<td>8.0 (0.1)</td>
</tr>
<tr>
<td>Specific Conductivity (µS/cm)</td>
<td>558 (2)</td>
<td>556 (2)</td>
<td>563 (2)</td>
<td>593 (2)</td>
</tr>
<tr>
<td>Median pH</td>
<td>7.2</td>
<td>7.4</td>
<td>7.6</td>
<td>7.4</td>
</tr>
<tr>
<td>Average Substrate Composition (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Silt</td>
<td>6</td>
<td>82</td>
<td>82</td>
<td>75</td>
</tr>
<tr>
<td>Sand</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>19</td>
</tr>
<tr>
<td>Gravel</td>
<td>59</td>
<td>4</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>Cobble</td>
<td>23</td>
<td>5</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Boulder</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Woody Debris</td>
<td>10</td>
<td>5</td>
<td>6</td>
<td>5</td>
</tr>
</tbody>
</table>

Total Female Fountain Darters | 127 | 144 | 141 | 119
FIGURE 1. Flow (m$^3$/s) throughout the study period by site: (Top) Site 4-City Park, San Marcos River; Second from top: Site 3-New Channel, Comal River; Dotted line: Site 2-Old Channel, Comal River; Circles: Site 1-upper spring run, Comal River.
FIGURE 2.—PC analysis of habitat parameters for Fountain Darters collected across all sites (top), within site (bottom left), and across months (bottom right).
FIGURE 3.—Proportions of ovarian stages (top) and Gonadosomatic indices (bottom) for all ovaries by month throughout the study period. Letters above GSI means indicate significant difference and numbers in parentheses below points denote sample size.
FIGURE 4.—Gonadosomatic indices (left) and proportion of ovarian stage (right) by site, throughout the study period.
FIGURE 5.—Mean Gonadosomatic indices (left) and Batch Fecundity (right) by flow environment (site) within Optimum season (top, Jan-Apr), Tailing season (middle, May-Aug), and Leading Season (bottom, Sep-Dec). Numbers below site indicate mean flow in m$^3$/s for given season and capital letters denote site level significant difference.
APPENDIX SECTION

APPENDIX A

Cross section of late developing ovary with ripening oocyte in follicle cell.
APPENDIX A.—Cross section of late developing ovary showing ripening oocyte (upper left) and chorion width inside follicle cells (blue).
APPENDIX B

Ovarian stages of the Fountain Darter from Latent to Spawning conditions.
APPENDIX B. — Ovarian stages of Fountain Darters: Latent (i), Early Developing (ii–vi), Late Developing (vii–xi), and Spawning (xii).
APPENDIX C

Example of ripening oocyte and ova.
APPENDIX C.—Ripening oocyte (left) and ova (right). Widened chorion is present on both, however indentation is exclusive to ova.
REFERENCES


Texas Legislature. 1993. Senate Bill 1477, 73rd session, Austin.


