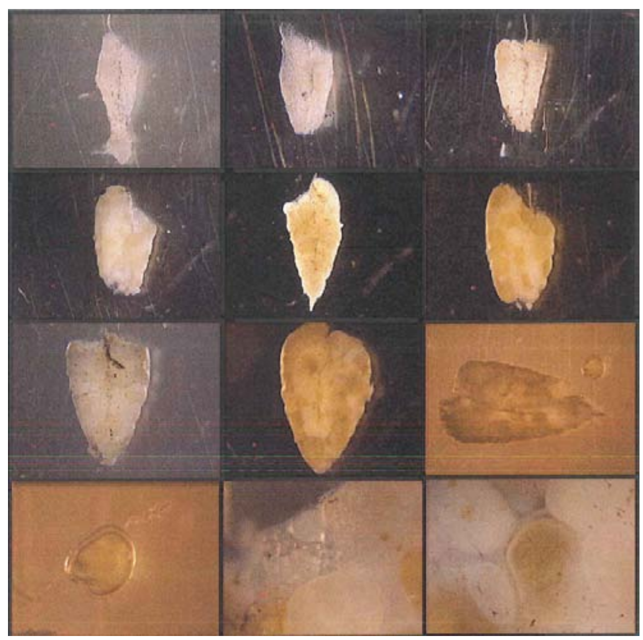
EFFECTS OF LOW FLOW ON FOUNTAIN DARTER REPRODUCTIVE EFFORT

HABITAT CONSERVATION PLAN (HCP) 2014 APPLIED RESEARCH



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FINAL REPORT

October 2014

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1.0 INTRODUCTION

The Edwards Aquifer Habitat Conservation Plan (HCP) is founded on long-term biological goals for the covered species that inhabit the Comal and San Marcos springs/river ecosystems (EARIP 2011). To support the long-term biological goals, flow management objectives (flow regimes) were established that are presumed to be protective of the threatened and endangered species in these systems. The low-flow conditions (discharge and extended durations) incorporated in the HCP flow regime and projected to occur during severe drought have occurred very infrequently (or not at all) during the historical record. Consequently, complete testing of ecological response(s) to these conditions in the wild is unlikely. Therefore, testing of simulated conditions in laboratory and/or field environments is mandatory to address HCP unknowns.

Section 6.3.4 of the HCP lays out the path forward for answering key questions and filling in data gaps to test assumptions and ultimately assist with management decisions. The focus in 2013 was on addressing several key questions surrounding physical habitat and food source responses both related to the federally-listed endangered Fountain Darter *Etheostoma fonticola*. In 2014, three additional applied research projects focused on the Fountain Darter were conducted. This report focuses on the effects of low flow on Fountain Darter fecundity.

Reproductive success of slackwater and benthic fishes is reduced under low flow conditions, attributed to greater variability in physical habitats and to increases in organic substrates (Schlosser 1982, Falke et al. 2010). As flows decrease, aquatic vegetation (e.g., physical habitat) proliferates but not homogeneously among plant taxa (Bunn and Arthington 2002, Riis and Hawes 2002). Physical habitat alteration, such as changes in the plant community, can reduce foraging efficiency and alter spatial distributions and habitat quality (Dibble et al. 1997, Dibble 2010). Modified vegetative structural complexity (i.e., low-growing vs. tall-growing, sparse vs. dense macrophytes) in conjunction with accumulation of organic sediments under a declining hydrograph, can limit spawning and nursery habitats of stream fishes, especially those that attach eggs to plants or substrates (Dibble et al. 1997). As such, studies documenting the effects of the alteration of flow regime within associated habitats of the Fountain Darter are of extreme importance to the HCP.

Reduction in base flow restricts the amount of available habitat for spring-associated fishes (Hubbs 1995), likely fragments habitats and impedes movement (Dammeyer et al. 2013), decreases Fountain Darter reproductive success (Brandt et al. 1993, Bonner et al. 1998), and increases intraspecific competition (Araujo 2012) and gill-parasite mortality (McDonald et al. 2006, Tolley-Jordan and Owen 2008). Modeling suggests that reducing the 19-year mean base flow conditions (184 cfs) to 58 cfs (32% of current base flow) would noticeably reduce Fountain Darter populations in the San Marcos River (Mora et al. 2013). Empirical evidence supports this prediction, given that Fountain Darters were considered extirpated from the Comal River in 1973, attributed to cessation of spring flows though possibly affected by rotenone treatment to remove non-native fishes in the 1950s, and a catastrophic flood in 1972, prior to documenting the extirpation in the mid-1970s (Schenck and Whiteside 1976).

Given that low flow conditions will alter the habitats of the Fountain Darter, we predict that reproductive effort within levels of vegetated to non-vegetated habitat types and within high to low discharge environments will be reduced, respectively. To test this prediction, we first established a baseline in Fountain Darter reproductive readiness among a gradient of flow regimes and among vegetation types. Objectives of this study were to quantify elements of Fountain Darter reproduction (gonadal recrudescence, ovarian development) among available flow gradients ranging from 5 to 120 cfs in the wild and among physical habitat types and substrates (open substrates, low-growing and tall-growing aquatic vegetation).

HCP Ecological Model Parameterization

The Fountain Darter fecundity study directly assessed the influence of flow and aquatic vegetation on Fountain Darter reproduction. Type and/or structure of aquatic vegetation are key components of Fountain Darter habitat in the HCP Ecological Model while discharge is a driving variable. Information generated from this work could provide direct measurements of reproductive success and expenditure for Fountain Darters throughout the year. Although the report puts forward parameters (reproductive effort by month, flow, and vegetation type) for consideration in model parameterization, it is emphasized that specific use of any of the 2014 applied research will be determined by the HCP ecosystem modeling team with guidance from the HCP Science Committee.

Recommendations for Future Applied Research

The Fountain Darter fecundity study was successful in evaluating the relationship in reproductive effort between discharge and vegetation type. However, it is acknowledged that this study only represents one partial year of data collection and did occur during an extended drought under total system discharge conditions not observed at Comal Springs since 1990. It is also described in the discussion section that unique habitat areas such as Spring Lake in the San Marcos system and Landa Lake in the Comal System may provoke different reproductive responses. As such, monthly sampling for an additional year with the collection of female Fountain Darters from existing sites and additional habitat areas is recommended.

Acknowledgments

The project team would like to acknowledge the U.S. Fish and Wildlife Service San Marcos Aquatic Resource Center (SMARC) scientists and staff. In particular, we thank Dr. Ken Ostrand and Dr. Tom Brandt for their guidance, assistance, patience and cooperation. As described throughout this report, Dr. Ostrand was integral in monthly field collection efforts and access was provided to SMARC laboratory facilities for all histology evaluations. We would also like to thank the HCP Science Committee for their timely input regarding approaches and methods for research activities.

2.0 DATA REVIEW AND AVAILABLE LITERATURE

For this reproductive assessment, the data review and literature compilation were performed for two major categories including baseline reproductive rates of Fountain Darters and habitats used by Fountain Darters for egg deposition. Each topic is addressed below.

Baseline reproductive rates of Fountain Darters: Fountain Darters are sexually dimorphic with males having distinct coloration in dorsal fins and short, pointed genital papillae and females having less intense pigmentation in dorsal fins and long, forked genital papillae (Schenck and Whiteside 1977). Sex ratios are slightly skewed toward males (1.39:1:00). Minimum length of reproduction is 24 mm in total length (Schenck and Whiteside 1977) at age 3.5 months (Linam et al. 1993) to 6 months (Brandt et al. 1993). Numbers of ova (ovulated oocytes within the ovary) are related to female length in darters with larger females producing more ova, though size of ova is independent of female length (Schenck and Whiteside 1977, Marsh 1986). Ova occur in female Fountain Darters year round, suggesting a protracted spawning season (12-months) but with reproductive peaks in late winter and late summer (Schenck and Whiteside 1977). Fountain Darters are batch spawners, producing a mean of 9 to 14.5 eggs per day during a 33 d period in a hatchery setting (Bonner et al. 1998) with 5 to 27 days, on average, between batches (Brandt et al. 1993). Eggs are released at water temperatures ranging from 3 to 30°C (Brandt et al. 1993) with optimum egg production ranging between 14 and 26° (Bonner et al. 1998, McDonald et al. 2007).

Habitats used by Fountain Darters for egg deposition: Fountain Darters are facultative phytophilic spawners (Simon 1999) depositing adhesive eggs on macrophytes (Strawn 1956, Phillips et al. 2011) but also on hard substrates lacking vegetation (Brandt et al. 1993). To date, Fountain Darters deposit eggs have been observed on *Rhizoclonium*, *Ludwigia*, *Sagittaria*, and *Zizania* (Phillips et al. 2011), but this is likely an incomplete list. Fountain Darters associate with a wide variety of vegetation, including *Riccia*, *Rhyzoclonium*, *Hydrilla*, *Ludwigia*, *Potamogeton*, *Sagittaria*, *Vallisneria*, *Hygrophila*, and *Cabomba* (Schenck and Whiteside 1976, Linam et al. 1993, Phillips et al. 2011, Alexander and Phillips 2012, Araujo 2012, Dammeyer et al. 2013) and areas without vegetation (Crowe and Sharp 1997, Araujo 2012, Behen 2013). Fountain Darters, in general, associate with slackwater and low velocity habitats, ranging in depths from < 0.5 m to 5 m with silt to cobble substrates (Behen 2013). Sister species within Subgenus Microperca (*E. microperca* and *E. proeliare*; Near et al. 2011) also are associated with slackwater to run habitats consisting of detrital terrestrial leaves, woody debris, and dense vegetation (Burr and Page 1978; Paine et al. 1981; Johnson and Hatch 1991).

3.0 MATERIALS AND METHODS

3.1 Field Collections

Sampling occurred monthly starting in January proceeding into August, 2014. Sample sites included City Park reach of the San Marcos River (Hays County, Texas) (Figure 1) and New channel, Old channel, and upper spring run reaches of the Comal River (Comal County, Texas) (Figure 2). Within each site, dip nets (16.5" hoop x 1/16" mesh) (Figure 3) and seines (2m x 1m x 1/16'' mesh) were used to capture female Fountain Darters > 24 mm in total length (TL). Immature (< 24 mm TL) and male Fountain Darters were promptly returned to the immediate area of capture. Females selected for laboratory analysis were placed in a lethal dose of MS-222 and preserved in a 10% solution of buffered formalin.

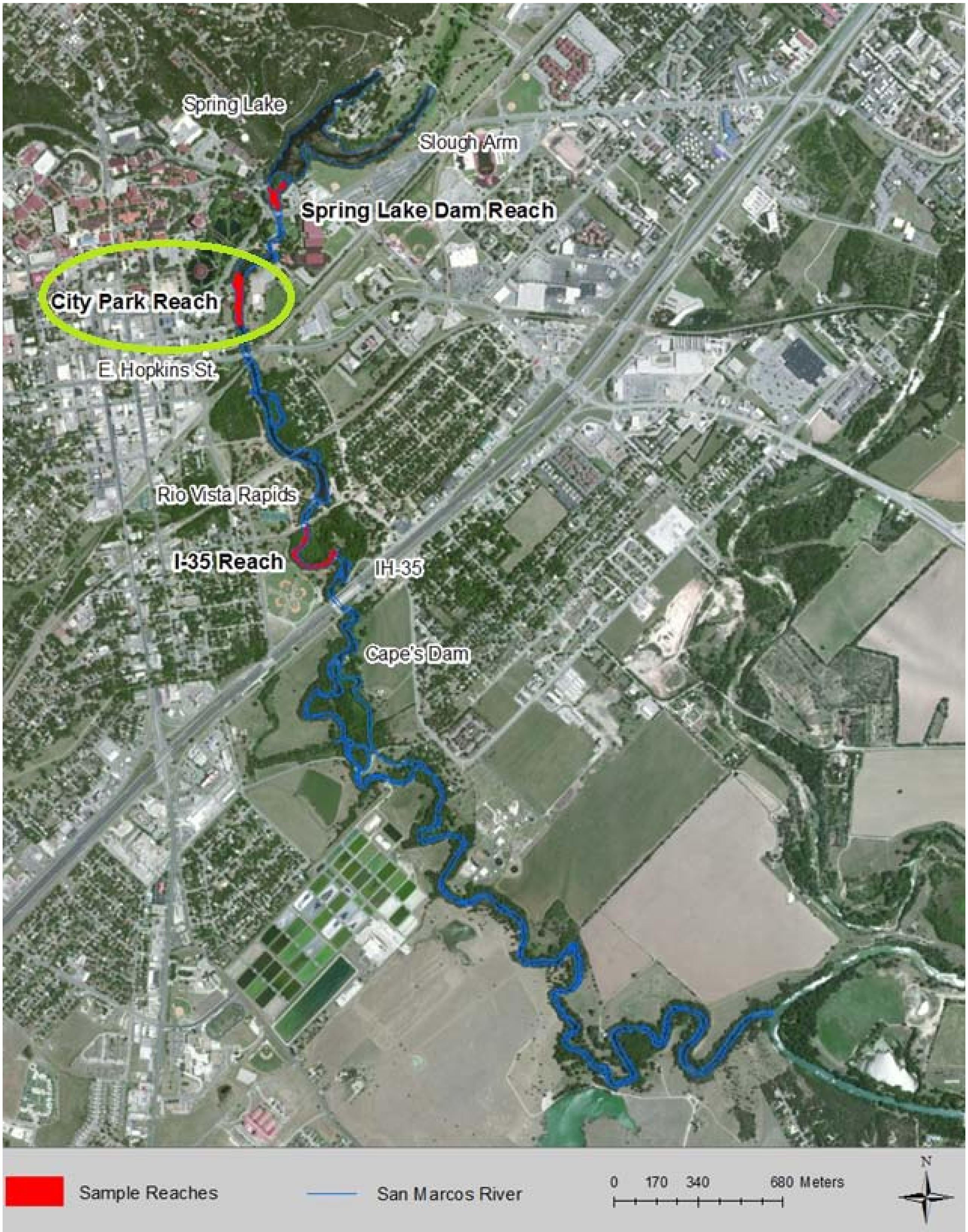

Figure 1. City Park sampling reach on the San Marcos River used for the Fecundity Study.

Figure 2. **Upper Spring Run, Old Channel, and New Channel study reaches on the Comal River.**

Figure 3. **Monthly field collections for mature female Fountain Darters in the Old Channel of the Comal River.**

Three vegetation types per site were sampled. Vegetation types were bare substrates (no vegetation), short vegetation (macrophytes < ½ of water depth) (Figure 4), and tall vegetation (macrophytes stands > ½ of water depth). Sample depth in meters (m) and vegetation height (m) were measured, along with visual estimation of the dominant vegetation. Current velocity was measured using a Marsh-McBirney Flo-mate™ portable velocity flow meter within vegetation and above (where applicable), and at 60% of the water depth for habitats without vegetation. Water quality was measured using a YSI 556 Multi-parameter System and included dissolved oxygen (mg/l), pH, temperature (˚C), and specific conductance (µS/cm). Percent substrate composition was visually estimated 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).

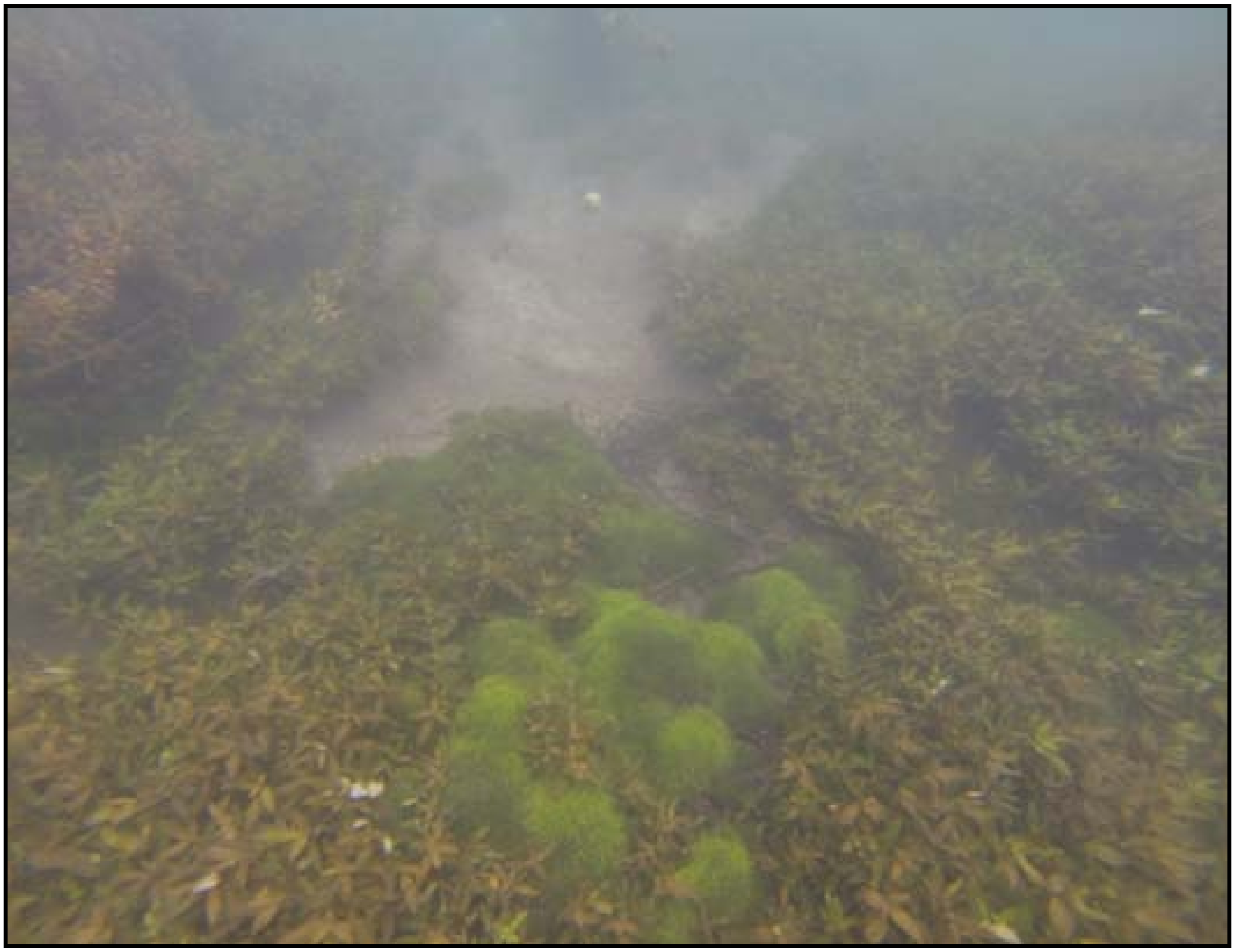

Figure 4. Examples of “short” aquatic vegetation sampled in during monthly field collections.

3.2 Laboratory Analysis

Samples were allowed to fix in solution for two weeks. Fish were transferred from formalin to 70% ethanol, total length was measured, and ovaries were excised. Gonadosomatic index (GSI) was estimated as the percent ratio of ovary to eviscerated body weight (liver, intestinal tract, and other viscera removed). Oocyte to ova maturation and ovarian stage was estimated using methodologies specific to darters modified from Heins and Baker (1989), Heins et al. (1992), and Heins (1995). Following recommendations provided by Brewer et al. (2008), a small sample of ovaries were selected from hatchery stock and conditioned to specific stages of development. These individuals were used for histology to confirm classification of ovarian stage. All laboratory analyses were conducted at the U.S. Fish and Wildlife Service San Marcos Aquatic Resources Center (SMARC), in San Marcos, Texas (Figure 5).

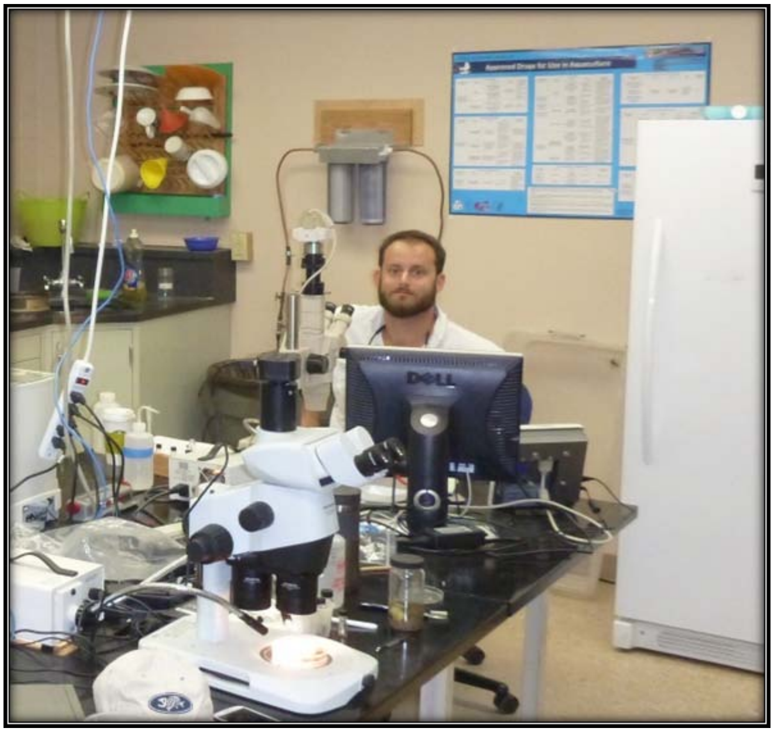

Figure 5. Laboratory analysis of ovary stage conducted at the SMARC.

Ovarian stage was separated into four distinct categories: pre-vitellogenic, early vitellogenic, late vitellogenic, and spawning (Appendix 1). Pre-vitellogenic ovaries appeared small, clear or translucent and were classified as latent (Heins 1995). Early vitellogenic ovaries were larger than latent, and opaque in appearance with a moderate amount of oocytes enlarged (Heins 1995). Late vitellogenic ovaries were greatly enlarged, and contained larger oocytes in the last stages of vitellogenesis or pre-ovulation (Heins et al. 1992; Heins 1995). Spawning ovaries contained ova distinguished by a small infold and enlarged chorion similar to descriptions provided by Schenck and Whiteside (1977) and Heins (1995).

3.3 Experimental Design and Data Analysis

The experimental design for this study involved the following response variables: Gonadosomatic index and percent of mature ovaries. The experimental unit was an individual female Fountain Darter and the following three treatments were tested:

Treatment 1: Flow regime (four levels)
Treatment 2: Vegetation type (three levels)
Treatment 3: Month (eight levels)

This resulted in an 8 x 4 x 3 = 96 design. Five replications per treatment were selected to control variability relating to batch spawning fishes (5 x 96 = 480 female Fountain Darters). Availability by site, month, and substrate type dictated the total number of female darters, so total catch was less than the anticipated 480.

Mean daily discharge (cubic feet per second; cfs) was obtained from USGS Stations (Comal River-Old Channel: 08168913; Comal River-New Channel: 08168932; San Marcos River: 08170500). Mean discharge was calculated via a transect method at Comal River-Upper Spring Run.

Gonadosomatic indices were calculated for all fishes by site and month and for Mature-Ripe ovaries only. Monthly differences in GSI-All Ovaries were assessed across all sites with one-factor ANOVA ($\alpha = 0.05$), followed by post-hoc Fisher's Least Significance Difference, to determine if reproductive effort is homogenous across months as expected for a year-round spawning fish. Mature-Ripe ovaries were selected as the most sensitive indicator of reproductive effort, because this stage of late vitellogenic ovary is the most advanced without ovulation and egg release (Heins and Baker 1989). Hence, weights of Mature-Ripe ovaries are not influenced by prior release (minutes to hours) of ovum. Differences in GSI-Mature-Ripe Ovaries (dependent variable) by site (categorical independent variable; represents differences in discharge) and vegetation type (categorical independent variable) were assessed with a two-factor ANOVA, followed by post-hoc Fisher's Least Significance Difference test. Site*Vegetation interaction term was not significant ($P = 0.67$) and dropped from the linear model.

4.0 RESULTS AND DISCUSSION

Mean discharge (± 1 SD) ranged from 3.3 (2.7) cfs in the Comal River-Upper Spring Run to 145 (30.2) cfs in the San Marcos River-City Park (Figure 6). Discharge during the period of observation decreased in the Comal River-Upper Spring Run, stayed fairly constant in the Comal River-Old Channel, decreased in the Comal River- New Channel, and slightly decreased in San Marcos River-City Park, though supplemented with multiple pulse flow events. Mean water temperature (calculated at location of each fish taken) ranged between 21.5°C at San Marcos River-City Park to 24.2°C at Comal River-Upper Spring Run (Table 1). Minimum water temperatures ranged between 18.6°C at San Marcos River-City Park to 20.3°C at Comal River-Old Channel, and maximum water temperature ranged between 22.7°C at San Marcos River-City Park to 29.6°C at Comal River-Upper Spring Run. The lowest dissolved oxygen level (4.5 mg/l) was observed at Comal River-Upper Spring Run. Fish were taken from a variety of substrate types, through predominantly from gravel at Comal River-Upper Spring Run or from silt at the other three sites. Mean relative vegetation heights ranged from 15 to 31% of water depth for short vegetation and 57 to 71% of water depth for tall vegetation.

Among all sites and for 335 Fountain Darters, four stages of ovarian development were found from January through August 2014, except latent ovaries were absent in June 2014 (Figure 7). Occurrences of spawning ovaries and late vitellogenic ovaries from January through August indicate egg release throughout the study period. However, proportions of spawning ovaries and late vitellogenic ovaries decreased through time. Fish with spawning ovaries and late vitellogenic ovaries comprised >50% of the breeding population from January through July and <25% in August. Reproductive effort, as measured by GSI-All Ovaries, differed ($P < 0.01$) among months with a peak in March, elevated in January, February, April, and June, and decreased in May, July, and August. Hence, reproductive effort was not constant through time.

Among individual sites, GSI-All Ovaries generally decreased from January through August (Figure 8). Occurrence of spawning ovaries indicated egg release during each month and site, except in April and August at Comal-Upper Spring Run, Comal-Old Channel, and Comal-New Channel.

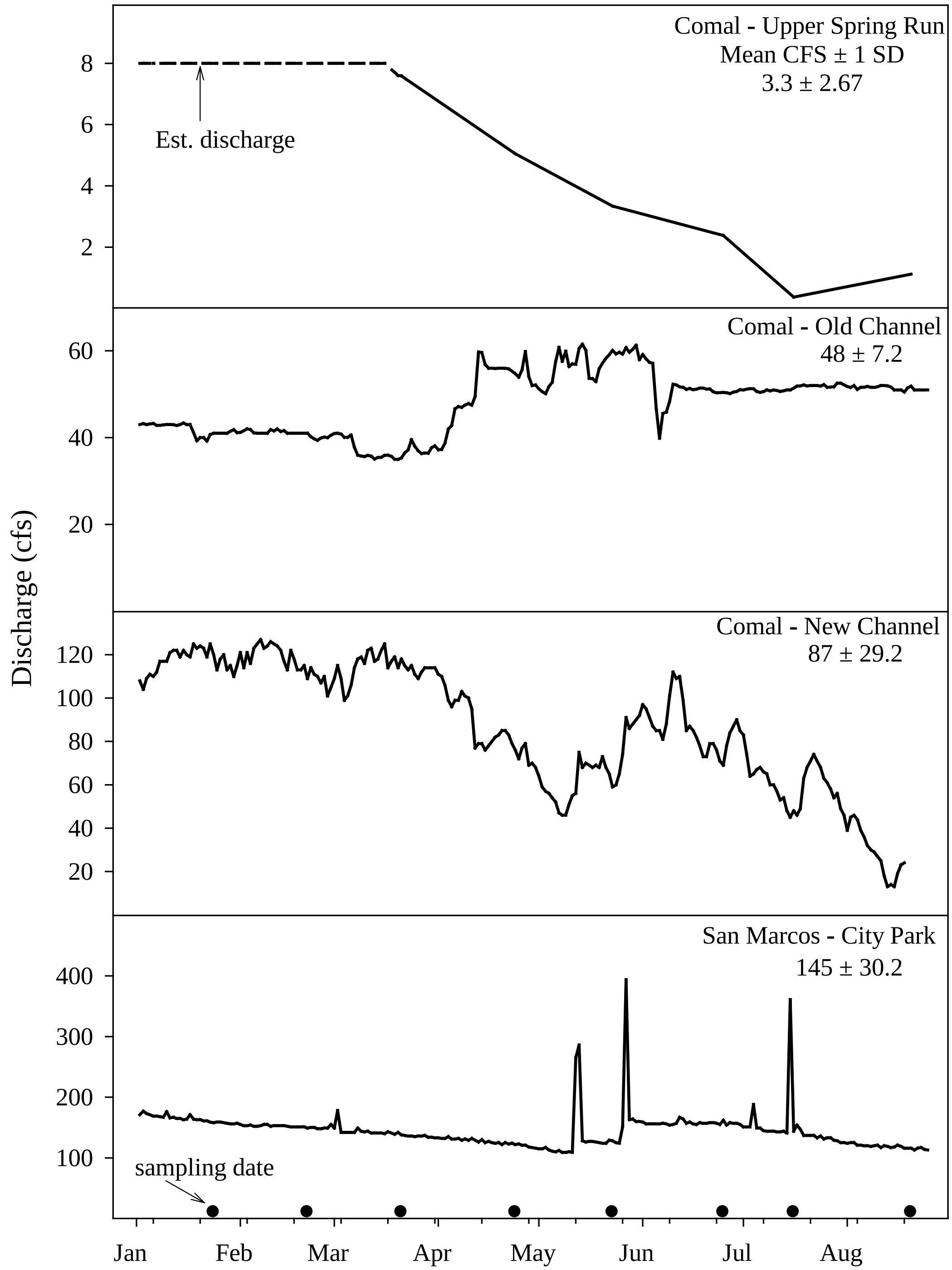

Figure 6. **Hydrograph (mean daily discharge) for each study site and collection dates of Fountain Darters taken. Comal River-Upper Spring Run hydrograph was calculated via transect method on the day of sampling.**

Table 1. Water quality measurements among four sites and across months.

River	Comal River			Comal River		
Site	Upper Spring Run			Old Channel		
Mean Discharge (cfs^{-1})	3.3			48		
	Mean (SE)	Min	Max	Mean (SE)	Min	Max
Temperature (˚C)	24.18 (0.2)	19.5	29.6	22.7 (0.2)	20.3	24.3
pH	7.33 (0.04)	7	7.8	7.49 (0.02)	7.28	8.19
Dissolved Oxygen ($mg*l^{-1}$)	7.97 (0.2)	4.54	11.86	8.33 (0.14)	6.4	12.8
Specific Conductance ($\mu S*cm^{-1}$)	558 (2.2)	491	590	551 (2.8)	516	595
Habitat Type	Bare	Short	Tall	Bare	Short	Tall
	mean (SE)	mean (SE)	mean (SE)	mean (SE)	mean (SE)	mean (SE)
Depth (m)	1 (0.05)	0.68 (0.03)	0.77 (0.03)	1.0 (0.06)	0.68 (0.04)	0.71 (0.03)
Current Velocity ($m*s^{-1}$)	0 (0.0)	0.02 (0.00)	0.01 (0.01)	0.24 (6.7)	0.06 (0.00)	0.09 (0.01)
Aquatic Vegetation Height (m)	-	0.09 (0.01)	0.49 (0.03)	-	0.19 (0.02)	0.37 (0.02)
Relative Height Index	-	0.15 (0.02)	0.67 (0.04)	-	0.29 (0.03)	0.57 (.04)
% Woody Debris	11 (7.62)	0	0	28.4 (10.1)	0	0
% Substrate						
Silt	13 (5.4)	6.7 (1.9)	12.9 (4.8)	12.5 (7.2)	96.9 (2.6)	98.2 (0.58)
Sand	1 (0.6)	1.7 (0.77)	2.3 (2.3)	20.6 (7.9)	0.13 (0.13)	0
Gravel	58 (10.3)	65.6 (5.5)	55.9 (6.5)	18.4 (7.1)	2.56 (2.1)	0
Cobble	24 (8.8)	26.0 (5.9)	29.0 (5.6)	40.6 (9.6)	0.38 (0.38)	1.178 (.57)
Boulder	2 (1.7)	0	0	7.8 (3.0)	0	0

Table 1 (continued). Water quality measurements among four sites and across months.

River	Comal River			San Marcos River		
Site	New Channel			City Park		
Mean Discharge (cfs^{-1})	81			138		
	Mean ± (SE)	Min	Max	Mean ± (SE)	Min	Max
Temperature (˚C)	22.8 (0.22)	18.8	24.9	21.5 (0.20)	18.6	22.7
pH	7.69 (0.02)	7.48	7.99	7.43 (0.04)	7.14	7.68
Dissolved Oxygen ($mg*l^{-1}$)	8.53 (0.14)	7.12	10.83	8.58 (0.16)	6.77	10.95
Specific Conductance ($\mu S*cm^{-1}$)	558 (2.8)	517	600	584 (3.9)	533	620
Habitat Type	Bare	Short	Tall	Bare	Short	Tall
	mean (SE)	mean (SE)	mean (SE)	mean (SE)	mean (SE)	mean (SE)
Depth (m)	0.44 (0.06)	0.84 (0.05)	0.72 (0.02)	0.47 (0.09)	0.85 (0.04)	0.74 (0.04)
Current Velocity ($m*s^{-1}$)	0.07 (0.02)	0.06 (0.01)	0.06 (0.01)	0.09 (0.03)	0.08 (0.02)	0.06 (0.01)
Aquatic Vegetation Height (m)	-	0.26 (0.03)	0.47 (0.02)	-	0.23 (0.02)	0.56 (0.06)
Relative Height Index	-	0.31 (0.03)	0.66 (.02)	-	0.29 (0.03)	0.71 (0.05)
% Woody Debris	0	0	0	0	0	0
% Substrate						
Silt	13.6 (7.0)	90.4 (4.5)	92.0 (3.4)	50 (29.0)	88.4 (5.5)	78.4 (7.2)
Sand	25 (6.7)	0	0	43.3 (29.6)	11.5 (5.5)	19.8 (6.6)
Gravel	56.8 (8.9)	6.0 (3.3)	7.3 (3.3)	6.7 (6.7)	0	1.8 (0.7)
Cobble	4.6 (1.3)	0	0.71 (0.3)	0	0	0
Boulder	0	0	0	0	0	0

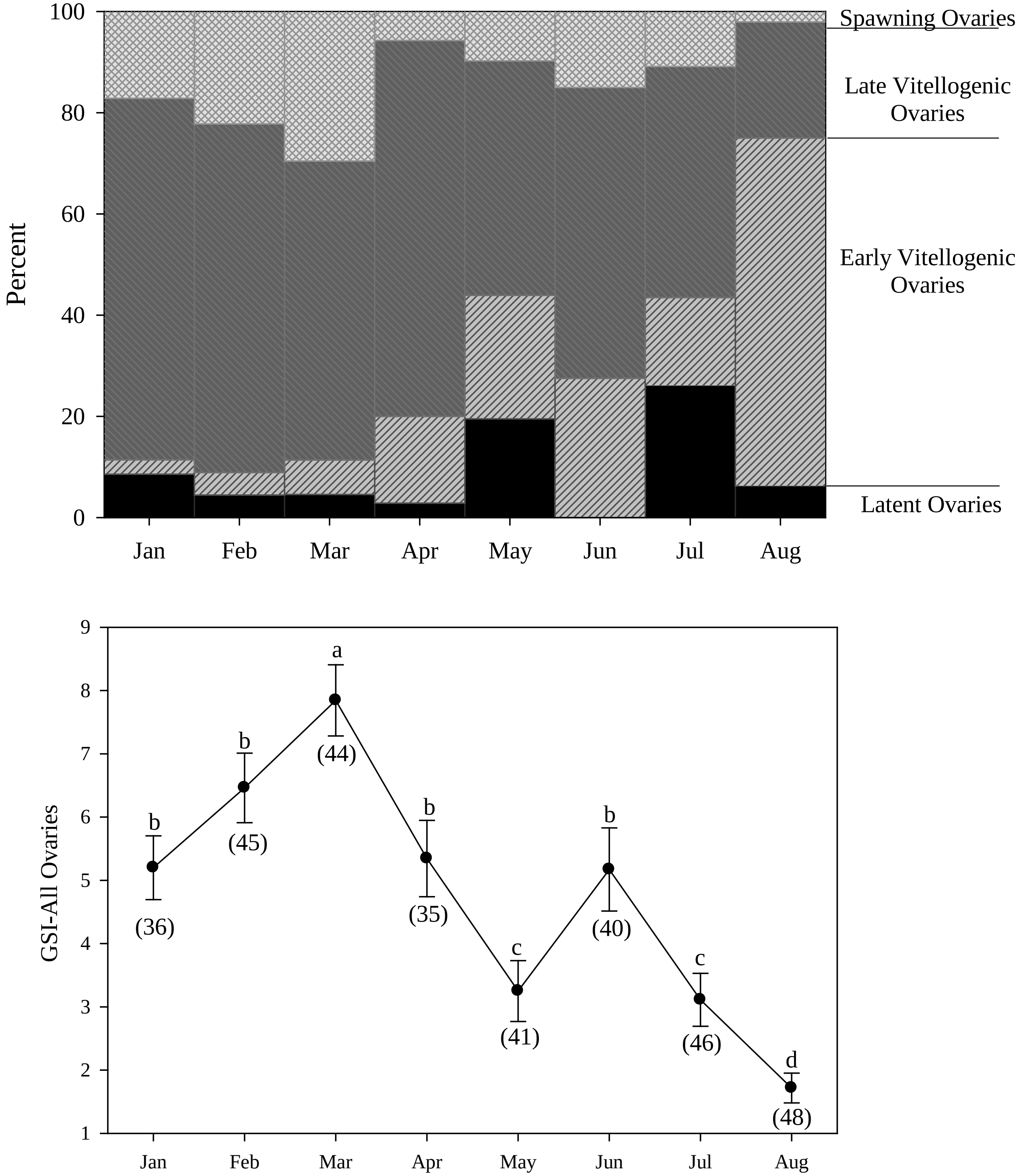

Figure 7. **Ovarian stages for all Fountain Darters (N = 355, upper panel) and gonadosomatic indices for all female Fountain Darters (lower panel) taken from four sites on San Marcos River and Comal River. Parenthetical numbers represent N of fishes per month. Same lower case letter represent no significant difference between months.**

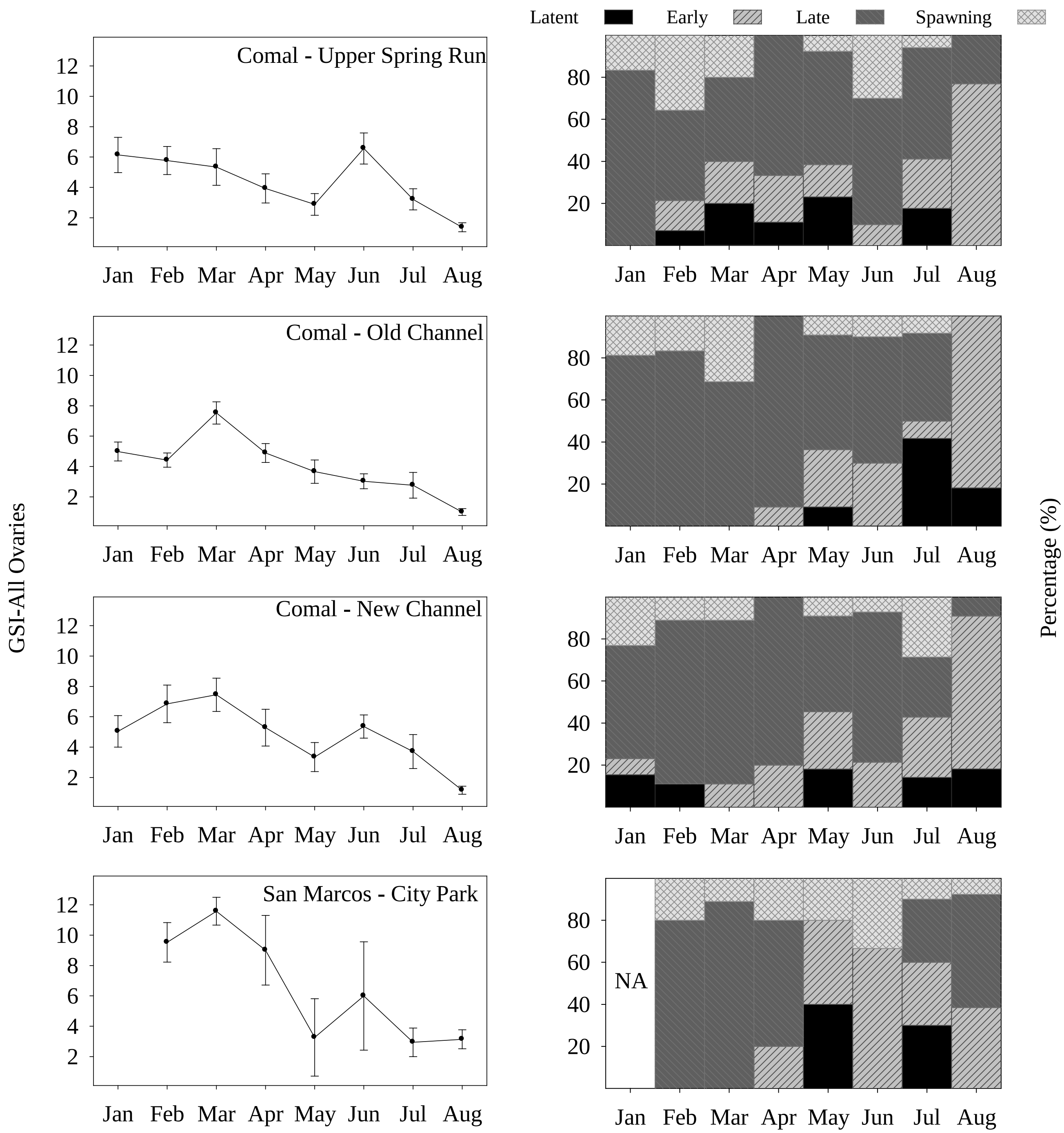

Figure 8. **Gonadosomatic indices for fishes >24 mm in TL (size of sexual maturity) among four sites (left panel) and associated ovarian stages (right panel).**

Gonadosomatic indices-Mature-Ripe Ovaries differed among sites and vegetation type ($P<0.01$) (Figure 9). Mean (± 1 SE, N) of GSI (Ripe-Mature Ovary) taken from San Marcos River-City Park was 8.4 (0.67, 21) and was greater than those taken from Comal River-New Channel (7.0 ± 0.38, 41), Comal River-Old Channel (6.1 ± 0.34, 39), and Comal River-Upper Spring Run (6.5 ± 0.39, 24). Mean of GSI (Ripe-Mature Ovary) taken from tall vegetation was 7.5 (0.35, 60), did not differ from those taken from bare substrate (7.0 ± 0.47, 12), and was greater than those taken from short vegetation (6.1 ± 0.29, 53).

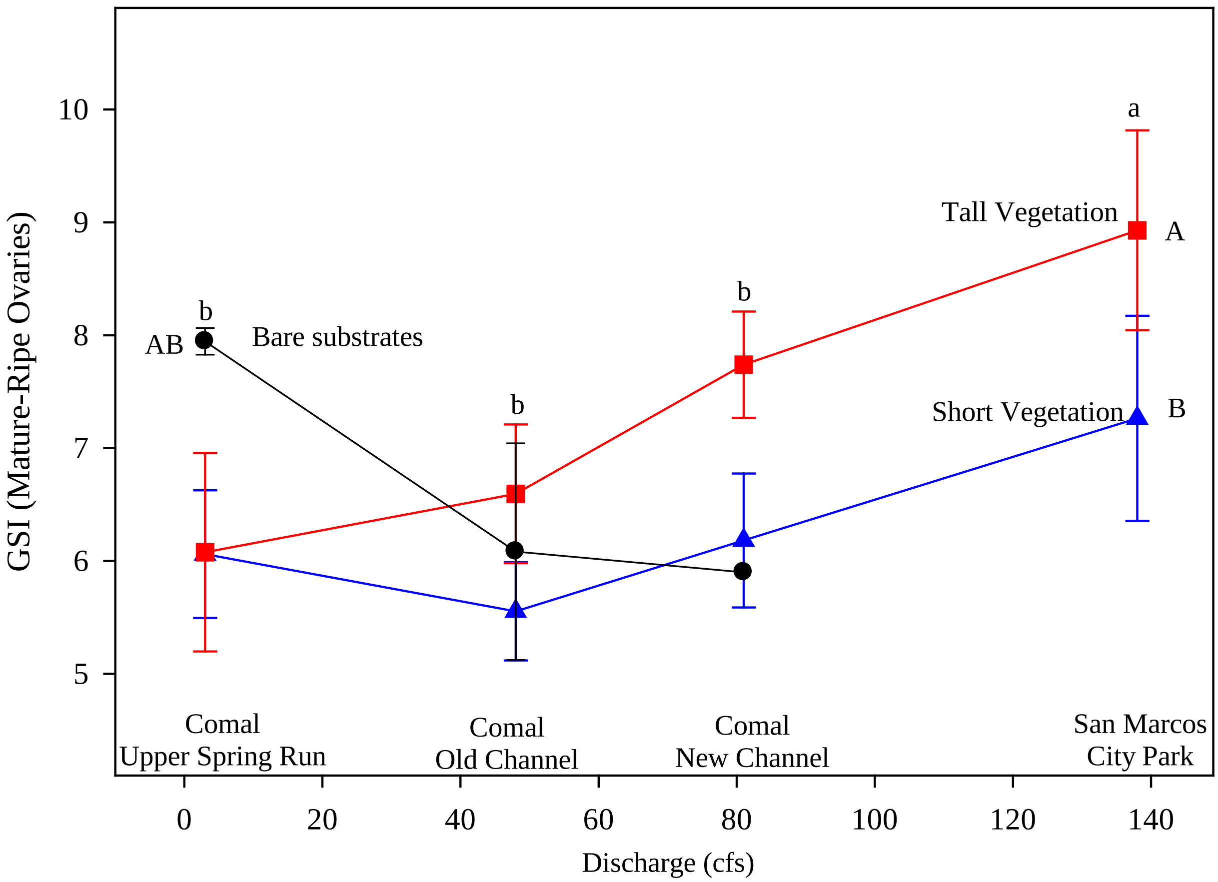

Figure 9. **Gonadosomatic indices for fishes with Mature-Ripe Ovaries by site and mean daily discharge and by vegetation type. Same upper case letters represent no significant vegetation type effect. Same lower case letters represent no significant site effect.**

Initial predictions on the relationship between reproductive effort and discharge were partially supported. Reproductive effort, as measured by GSI-Mature-Ripe Ovaries, was greater within greater discharge environments of the San Marcos River (mean discharge = 145 cfs). However, differences in reproductive effort were not detectable among discharges ranging from 3.3 to 87 cfs in the Comal River. Furthermore, spawning, as measured by occurrence of Spawning Ovaries, occurred at <1 cfs in Comal River-Upper Spring Run in July 2014. Therefore, differences in spawning among flow gradients were not detected under conditions encountered in 2014, whereas amount of energy invested into reproduction is dependent on discharge at levels >87 cfs.

Study results herein differ slightly than the results reported by Schenck and Whiteside (1977). Schenck and Whiteside (1977) reported peaks in reproductive effort as greater proportions of females containing mature ova in February and March and again in July and August. Conversely, we found a general decrease in reproductive effort from Spring through Summer. Our study results, however, are consistent with reproductive efforts reported spawning patterns in other spring-associated minnows (McMillan 2011) and spring-associated darters (Folb 2010). In addition, our results are consistent with field observations within the San Marcos and Comal Rivers (BIO-WEST 2014a, 2014b). Small Fountain Darters (5 – 15mm, <60 days old; Brandt et al. 1993) were captured in the San Marcos River-City Park during dip netting events 23 of the 47 events (49%) since 2000 with most occurrences noted during the Spring (Figure 10). Small Fountain Darters were taken more often (44 of 47 events; 94%) in Spring Lake (Figure 11) than in San Marcos River-City Park, but higher proportions were again found in the Spring. In the Comal River, similar patterns are evident: New Channel (46% of samples contained small Fountain Darters), Old Channel (79%), Upper Spring Run (71%), and Landa Lake (90%) which again documents differences among sites. However, as with the San Marcos River, peaks in the Comal system were most evident in the Spring at all stations.

Initial predictions on the relationship between reproductive effort and vegetation type were largely unsupported. Reproductive effort was greater in tall vegetation at Comal River-Old Channel, Comal River-New Channel, and San Marcos-City Park. Reproductive effort was greatest on bare substrates in Comal River-Upper Spring Run, likely attributed to limited amounts of vegetation within the site.

Collectively, Fountain Darters reproduce for at least eight months (January – August) but reproductive effort is not equal among months or among sites (discharge). Mechanisms underlying reduced reproductive energy at discharges <145 cfs and in tall vegetation are unknown at this time. Density-dependent mechanisms, such as prey availability and Fountain Darter densities, are potential factors regulating reproductive investment. Information on food items consumed is available for Fountain Darters collected during this study and could yield insight into potential diet differences among sites and vegetation types, but the information has yet to be quantified. Additionally, measures of Fountain Darter densities are available and will be evaluated against reproductive investment at a later date.

Density dependent mechanisms influencing reproductive effort (investment and seasonality) have potentially interesting links to quality of habitats via field observations. As noted above, occurrences of small Fountain Darters are more frequent in Landa Lake and Spring Lake (>90%

occurrence among samples) than in Old Channel and Upper Spring Run (71 – 79%) or San Marcos-City Park and New Channel (<50%). Though reproductive investment appears to be higher in San Marcos-City Park, the greater frequency of small Fountain Darters year round at Upper Spring Run and Old Channel suggest extended spawning. Comparisons between reproductive investment and spawning are potentially useful as an indicator of habitat quality.

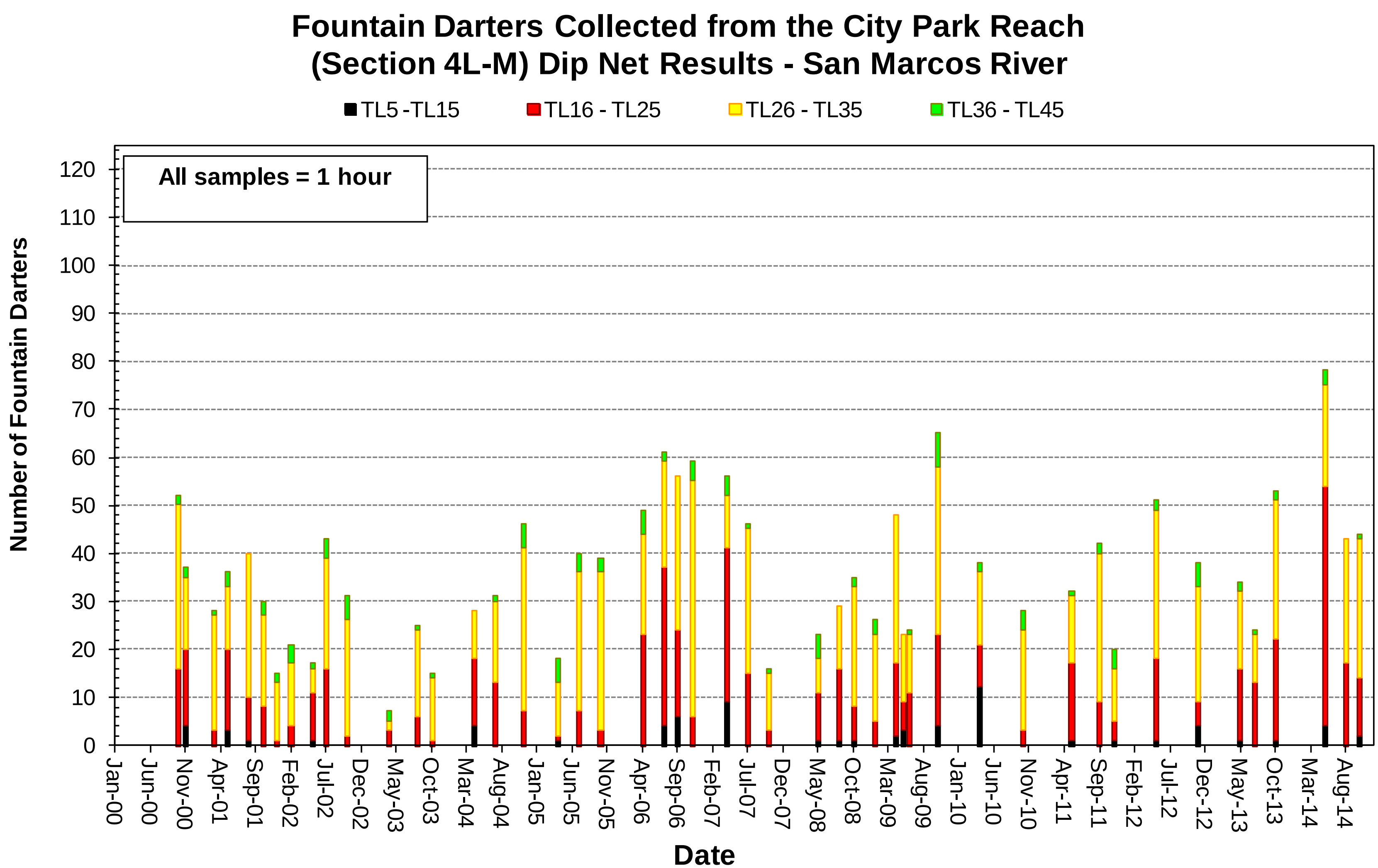

Figure 10. **Fountain Darter dip net results over time from City Park of the San Marcos River.**

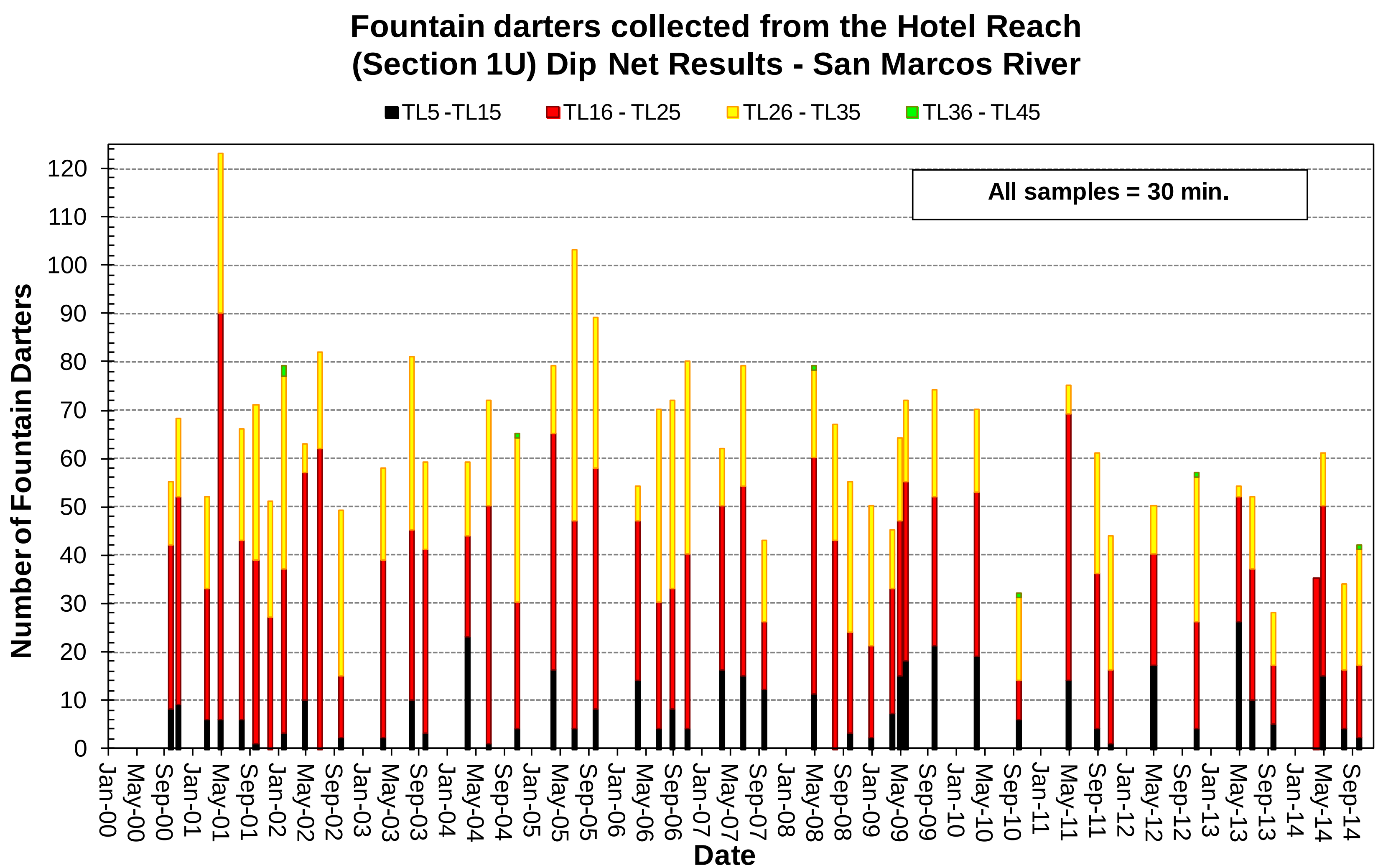

Figure 11. **Fountain Darter dip net results over time from Spring Lake of the San Marcos River.**

5.0 CONCLUSIONS

Fountain Darters are reported to be year round spawners. Evidence to date supports this but spawning effort is not equal among all months. Decreasing effort occurs during the summer months. Given the abiotic conditions recorded at each site, we hesitate to attribute this decrease to water year (below average flow this year) conclusively until comparable data are collected during an average or above average flow year. Reproductive effort differed among a flow gradient ranging between 3.3 and 145 cfs but only with marginal differences in GSI. Spawning did not cease across the flow gradient. In addition, vegetation type was associated with reproductive effort.

Mechanisms to explain the observed pattern are still being explored but likely include results of physical (structural components of vegetation) or biological (density-dependent) processes, such as amount of food available or number of conspecifics in the area. The relationships among physical and biological mechanisms and flow could offer insight on how flow indirectly affects Fountain Darter reproduction.

For the HCP Ecological parameterization, estimates of reproductive effort by month, flow, and vegetation type can be used to improve reproduction estimates in the model. Currently, the model is using water temperature as the primary determinant of reproduction. Information provided herein offers additional options to refine reproductive parameters in the final model.

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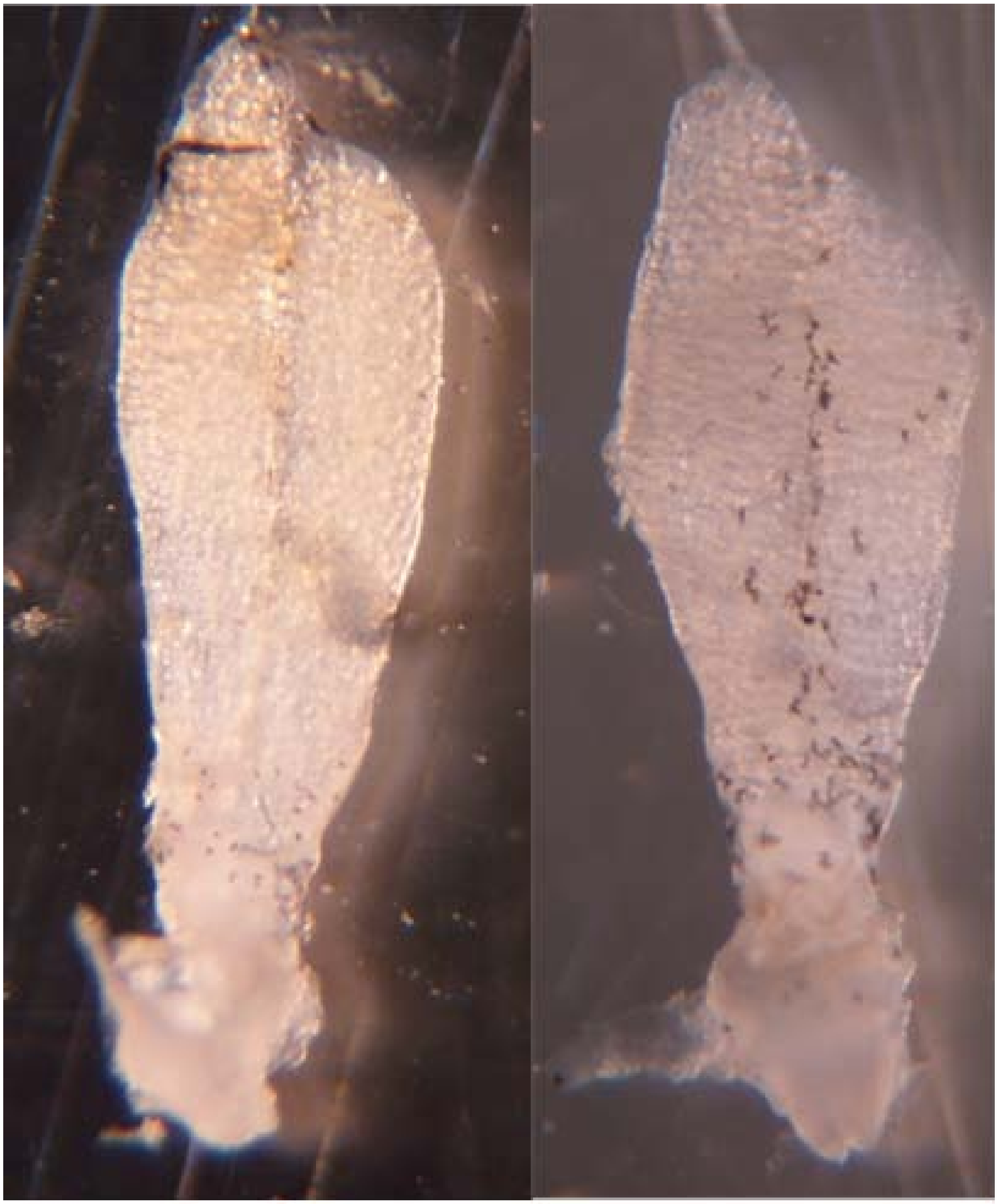
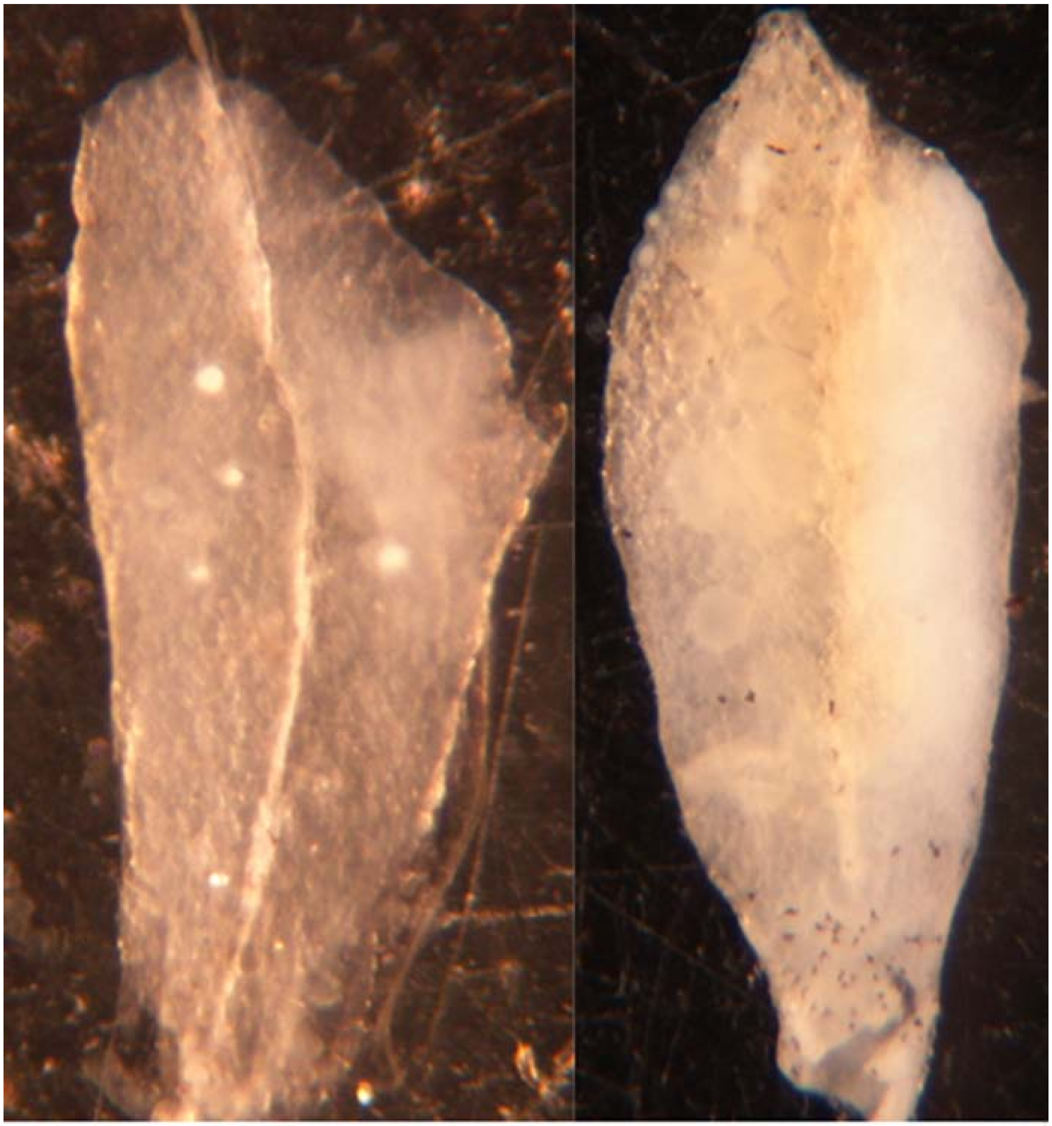
Appendix 1. Stages of ovarian and oocyte development in Fountain Darters taken from January through August 2014 among four sites on the San Marcos and Comal rivers.

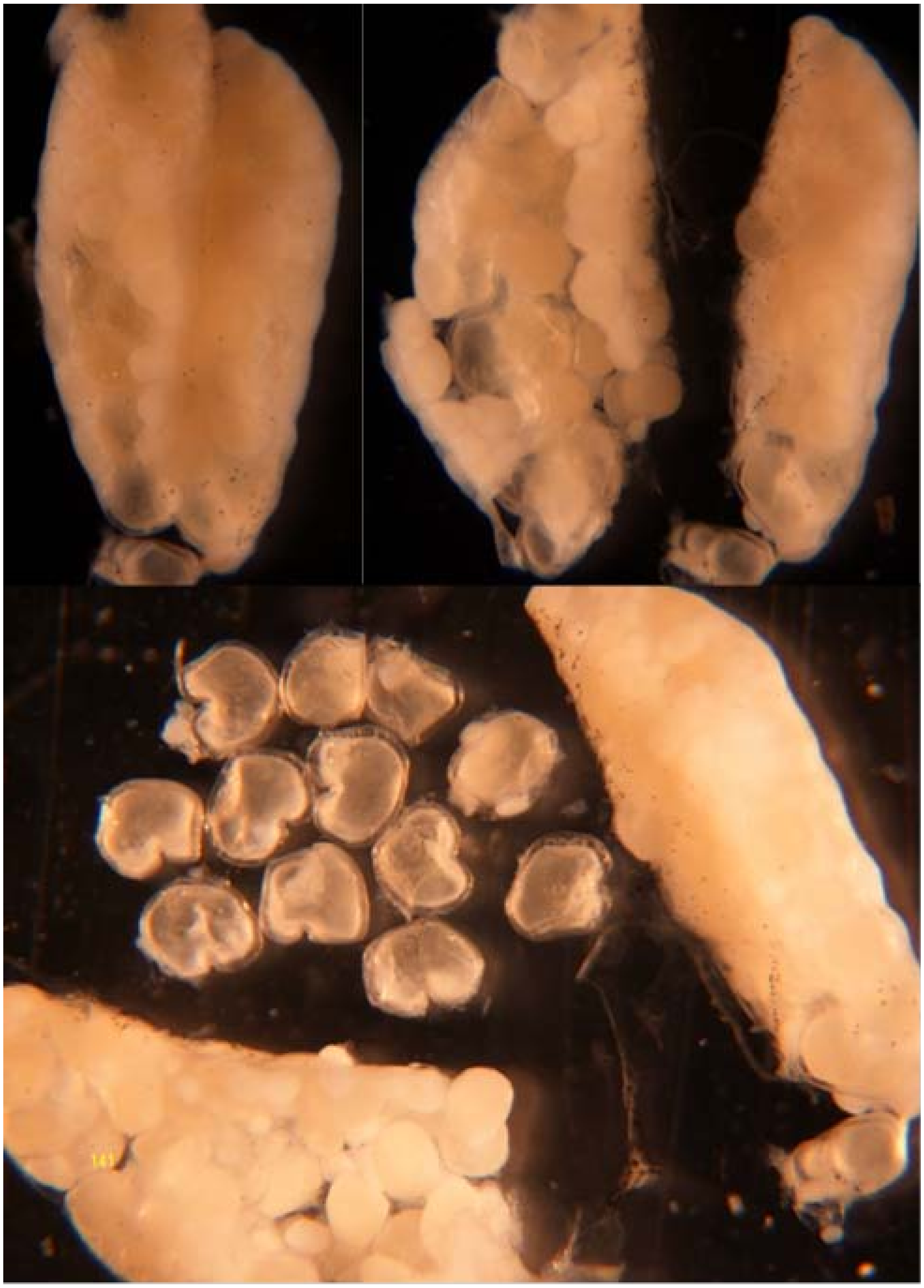


Pre-vitellogenic Ovaries; latent condition, semi translucent. Beginning stage of female Fountain Darter sexual maturity.



Early Vitellogenic Ovaries; beginning of vitellogenesis (yolk loading). Ovaries opaque, moderate amount of oocytes enlarged.

Late Vitellogenic Ovaries; towards end stage of vitellogenesis (yolk loading). Ovaries mostly opaque and enlarged. Oocytes noticeably engorged with yolk, chorion separation or thickening present but not overtly pronounced in non-ovulated clutch.



Spawning ovary; ovulated ovum present, ovaries enlarged. Ovum noticeably larger with yolk and chorion separation more distinct. Ovum with distinct infold on one side. Clutch size dependent upon relative time of capture from ovulation.

Ova; ovulated. Yolk appears as oil form; infolding present on one side. Chorion thickening greatly pronounced and viewed as translucent membrane surrounding the yolk (oil-like substance, opaque and off-white to yellow in color).