



**EFFECTS OF LOW FLOW
ON FOUNTAIN DARTER
FECUNDITY STUDY**

Literature Review and Proposed Methodology

PREPARED FOR:

HCP SCIENCE COMMITTEE

PREPARED BY:

BIO-WEST PROJECT TEAM - February 18, 2014



INTRODUCTION

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 (a physical habitat for fishes) proliferates but not homogeneously among plant taxa (Bunn and Arthington 2002, Riis and Hawes 2002). Therefore, changes in the plant community (i.e., low-growing/tall-growing, sparse/dense macrophytes) and accumulation of organic sediments under a declining hydrograph can limit spawning and nursery habitats of stream fishes, especially those that attached eggs to plants or substrates. Habitat association of the fountain darter is primarily benthic slackwater and low velocity habitats. Fountain darters inhabit no to dense vegetative cover in shallow and deep water habitats but associate with low growing vegetation (Schenck and Whiteside 1977, Alexander and Phillips 2012), which likely optimizes consumption of drifting and benthic prey and reproductive habitat while avoiding predators (Bergin 1996, Brandt et al. 1993, Phillips et al. 2011, Dammeyer et al. 2013). Given that low flow conditions will alter the physical habitats of the fountain darter, we predict that changes in physical habitats, especially low-growing and dense vegetation, will reduce the reproductive readiness and success of the fountain darter.

To test this prediction, we propose to establish a baseline in fountain darter reproductive readiness among a gradient of flow regimes and among vegetation type. Objectives of this study are to quantify elements of fountain darter reproduction (gonadal recrudescence, ovarian development, fecundity, and oocyte maturation) among available flow gradients ranging from 10 to 120 cfs in the wild and among physical habitat types and substrates (open substrates, low-growing and tall-growing aquatic vegetation).

Benefits to HCP Ecological Model

We are directly assessing the influence of flow and influence of vegetation on fountain darter reproduction. Flow and amount of vegetation are two key components of fountain darter habitat in the HCP Ecological Model, and information generated from this work will provide more accurate estimations on model parameters.

LITERATURE REVIEW

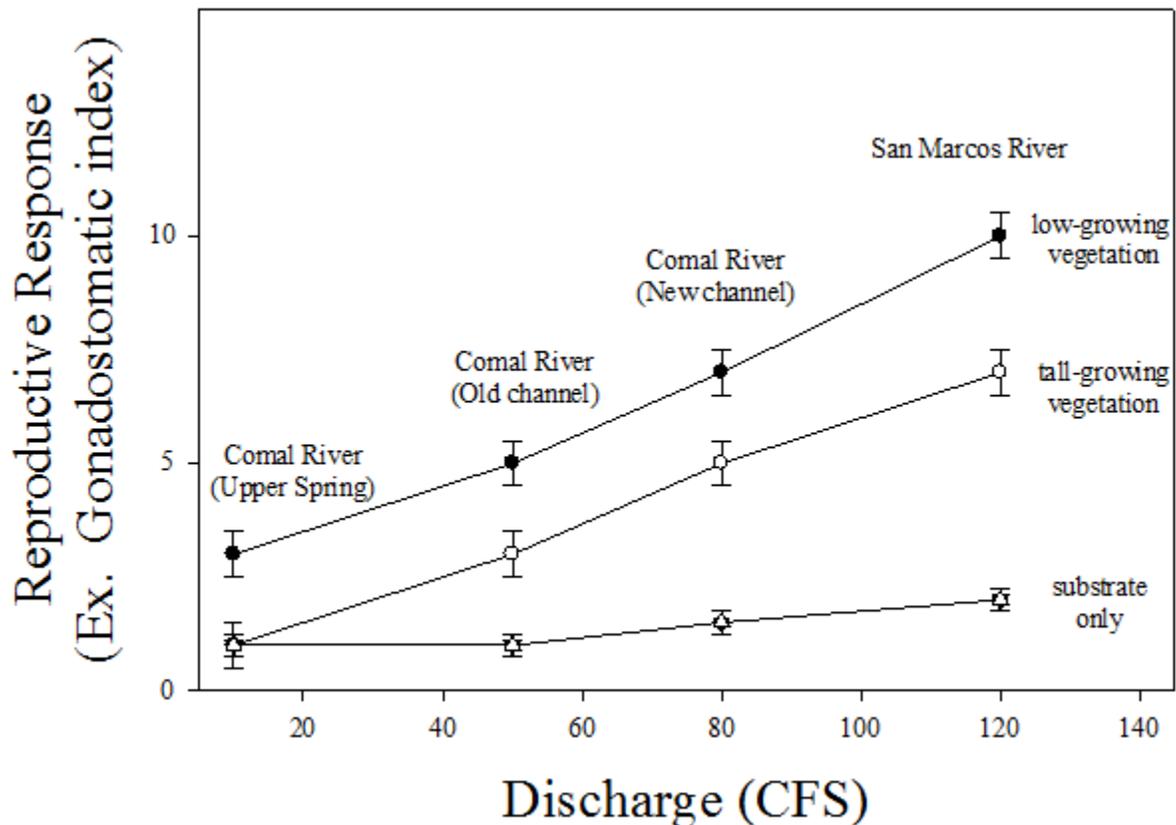
Baseline reproductive rates of fountain darters: Fountain darters are sexually dimorphic with males having distinct coloration in dorsal fins and short, pointed genital papillae and females having less intense pigmentation in dorsal fins and long, forked genital papillae (Schenck and Whiteside 1977). 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 darter eggs occur 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*, *Rhizoclonium*, *Hydrilla*, *Ludwigia*, *Potamogeton*, *Sagittaria*, *Vallisneria*, *Hygrophilia*, and *Combomba* (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).

Potential threats to fountain darter reproduction related to low flow conditions: Reduction in base flow reduces the amount of available habitat for spring-associated fishes (Hubbs 1995), and likely fragments habitats and impede 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). Fountain darters were considered extirpated from the Comal River in 1973, attributed to cessation of spring flows and a rotenone treatment to remove non-native fishes in the 1950s, a catastrophic flood in 1972, or a combination of the three events (Schenck and Whiteside 1976).

PROPOSED METHODS

As stated in the introduction, objectives of this study are to quantify elements of fountain darter reproduction among available flow gradients ranging from low flow (10 to 30 cfs; Upper Spring Run, Landa Lake), moderate flow (50 to 60 cfs; Old Channel-Comal River, and 80 to 100 cfs; new channel-Comal River), and high flow (100 to 120 cfs; San Marcos River) conditions and among physical habitat types and substrates (open substrates, low-growing and tall-growing aquatic vegetation). Study results should yield the following information: reproductive readiness of fountain darters among base flows ranging from 10 to 120 cfs and among substrates ranging in vertical depth from short (gravel and cobbles, no vegetation) to tall-growing vegetation. An example of potential results is presented in the following figure.



Sexually mature (>24 mm) female fountain darters will be harvested monthly between February and September 2014. On each collection trip and when available, up to 5 female darters will be taken from 3 to 5 areas of bare substrates (sand, gravel, cobble), short growing vegetation, and tall-growing vegetation within the San Marcos River and Comal River. Up to 60 female fountain darters will be taken on each collection trip. Fish will be anesthetized with MS-222 and preserved in 10% buffered formalin. In the laboratory, lengths and weights of each fish will be taken. Gonadosomatic index, ovarian stage, fecundity, and oocyte maturation will be determined for each fish following methodologies described specifically for darters by Heins and Baker (1989), Heins et al. (1992), and Heins (1995).

Monthly collections are necessary to quantify seasonality in fountain darter reproduction. In part, this work will support or revise spawning periodicity published by Schenck and Whiteside (1977). A more

refined methodology will be used to assess oocyte maturity (Heins and Baker 1989) than used by Schenck and Whiteside (1977). Also, monthly gonadosomatic indices might be used as a covariate in the experimental design of this study.

Experimental Design and Data Analysis

Response variables: Gonadosomatic index, number of ova, percent of mature ovaries

Experimental unit: individual female fountain darter

Treatment 1: Flow regime (four levels)

Treatment 2: Substrate type (three levels)

Treatment 3 (likely co-variate): Month (8 levels)

$$8 \times 4 \times 3 = 96$$

A minimum of five replications per treatment were selected to control variability relating to batch spawning fishes ($5 \times 96 = 480$ female fountain darters). A multi-factored MANOVA will be used to assess differences ($\alpha=0.05$) among treatment effects and response variables.

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