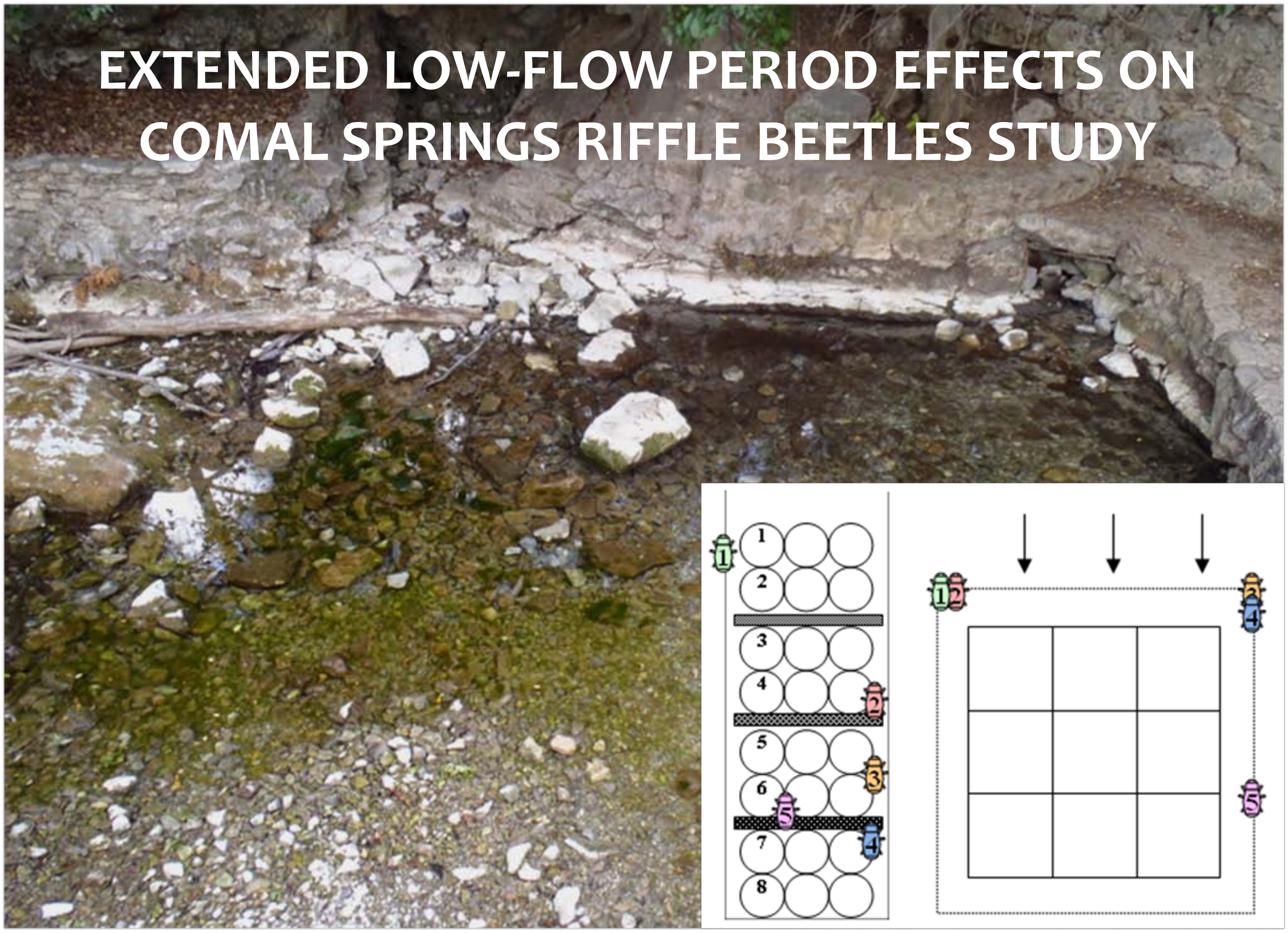
EXTENDED LOW-FLOW PERIOD EFFECTS ON COMAL SPRINGS RIFFLE BEETLES STUDY



Literature Review and Proposed Methodology

PREPARED FOR:

HCP Science Committee

PREPARED BY:

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Introduction

The Comal Springs riffle beetle (*Heterelmis comalensis*) was first collected by Linda Bosse in 1976 and later described from specimens found in the headwaters of the Comal River, New Braunfels, Texas (Bosse *et al.* 1988). All specimens collected by Harley Brown in 1977 were found in spring run 2 where water depth ranged from 2 to 10 cm, flowing over a gravel substrate into Landa Lake (Bosse 1979, Bosse *et al.* 1988). Adult Comal Springs riffle beetles have reduced hind wings, rendering them flightless, and are approximately 2 mm in length; females are slightly larger than males. Despite its affinity for springflow, the Comal Springs riffle beetle survived the drought of record (DOR) which resulted in cessation of flow from Comal Springs between 13 June and 3 November 1956 (Brune 1981).

The Habitat Conservation Plan (HCP) flow regime allows for periods of extended drying of the spring runs, and reduced surface habitat associated with the western shoreline and spring island areas (these areas are the strong hold for the Comal Springs riffle beetle in the Comal System). Although the HCP flow regime is not projected as severe on the minimum end as experienced in the DOR, the projected HCP extended periods of < 100 cfs are beyond what was observed in the DOR. Applied HCP research conducted in 2013 documented that aquatic vegetation was quite tolerant to the parameters tested (BIO-WEST 2013). If that finding holds in the wild, the Comal Springs riffle beetle may in fact be more of a sentinel species than the fountain darter (*Etheostoma fonticola*), and as such, understanding their tolerance is vital to making HCP Phase II decisions.

Over the past decade, Mr. Randy Gibson (USFWS), Mr. Chad Norris (TPWD), and researchers associated with the long-term biological monitoring program at Comal Springs have collected a wealth of information regarding Comal Springs riffle beetles. Building on the knowledge gained in both the field and laboratory over the years, the project team is providing the HCP Science Committee the following literature review and proposed methodologies for review and comment.

Literature Review

Although the term 'drought' is defined in a variety of ways in hydrologic literature (Humphries and Baldwin 2003; Gordon *et al.* 1997; Lake 2003; etc.), it is commonly understood that this term, while dependent on site specific studies, refers to times of low-flow that are outside of any normal seasonal flow variation (i.e. predictable variation during wet and dry seasons in tropical climates). Humphries and Baldwin (2003) defined drought as "an unpredictable low-flow period, which is unusual in its duration, extent, severity, or intensity". Periods of drought can alter an aquatic ecosystem in many ways. Decreasing water levels are often accompanied by loss of submerged vegetative habitats that are necessary for food and shelter (Ormerod *et al.* 1987), declining water quality (increased temperature, decreasing dissolved oxygen, etc.), and concentration of aquatic organisms in isolated pools, thus changing the normal community structure of benthic invertebrates (Flecker and Feifarek 1994).

Aquatic invertebrates, like the Comal Springs riffle beetle, are adapted to life in the water, thus it is expected that excessive decrease in flow and water levels associated with drought, and accompanying changes to normal physical and chemical properties of the water would impact both surface and subterranean aquatic life (Boulton 2003). In desert streambeds, drying has been observed to cause subsurface and surface habitats to become disconnected, which disrupted normal ecological processes in the streams by changing species composition in the stream (Valett *et al.* 1992). The composition of subsurface fauna has also been shown to change as drying occurred during a drought (Boulton and

Stanley 1995). The long-toed water beetle, *Postelichus immsi* (Coleoptera:Dryopidae), was observed moving upstream in a desert river in Arizona as downstream sections of the river were drying due to a drought in the region (Lytle, Olden, and McMullen 2008). Researchers found that the beetles were moving upstream at a rate faster than the downstream sections were drying, which indicated that the behavior was an adaptation that allowed them to escape local drought and move towards areas with higher flow. While the impact of drought and response by aquatic organisms has been studied for many surface taxa, and the stages of drought that are the most lethal for those populations have been well defined (Wright *et al.* 1994; Harrison 2000; Williams 1977; etc.), there are no studies that determine any critical level of drought at which subterranean or hyporheic taxa are most at risk (Boulton 2003).

H. comalensis is endemic to the Comal and San Marcos spring systems and inhabits the interstitial areas near spring upwellings of stream and lake beds (Cooke 2012). This species belongs to the subfamily *Elmidae*, which are known to be the most completely aquatic of all riffle beetles, with all life stages requiring aquatic habitat for survival (Brown 1987). These beetles do not have the adaptations that are normally associated with stygobionts, or obligate subterranean organisms, such as reduced eyes and pigmentation. However, this species' survival of the 7-year drought and the cessation of spring flow in 1956 indicate Comal Springs riffle beetles may be able to survive inside of the springs as well as in the interstitial habitat (Bowles *et al.* 2003). Knowledge about the food sources of *H. comalensis* is limited, however, it is accepted that this species, like other riffle beetles, are collector-gatherers and scrapers that feed primarily on detritus and algae (Elliott 2008). Microorganisms and decaying roots are also considered food sources for *H. comalensis* (USFWS 2007), which would suggest that individuals could survive inside of springs as far as tree roots extend into the aquifer. However, it is unknown how reliant these beetles are on decaying material (i.e. leaf litter) from their surface habitats. Adult *H. comalensis* utilize a plastron to obtain oxygen from the water, allowing them to remain completely submerged during their lifetimes as long as appropriate water pressure and nearly saturated dissolved oxygen (DO) levels are present. Reduced DO in an aquatic habitat as a result of decreased flow and increased temperature during a drought could, therefore, be deleterious to adult beetles. However, larvae are likely able to survive short periods of lower DO as they are not reliant on a plastron (Elliott 2008), which could also account for the species' survival of the 7-year drought in the 1950s.

In response to the hypothesis that *H. comalensis* have an adaptation that could allow them to potentially inhabit the springs during times of drought, the Edwards Aquifer Authority (EAA) sponsored a study in 2002 that observed *H. comalensis* and *M. pusillus*, another aquatic beetle in Comal Springs, and their response to varying flow regimes. The trials suggest that the beetles have a preference for flowing waters and will move towards a flow stimulus, which could be an adaptation that would allow the beetles to survive drought conditions by moving along spring upwellings towards flowing water within the aquifer (BIO-WEST 2002). No other studies present any knowledge about the movement of *H. comalensis* in response to drought, or their ability to survive in the springs as flows decrease and physical and chemical properties of water are altered. However, other species of Elmid beetles, such as *Macronychus glabratus* and *Stenelmis crenata*, have been known to survive several years in inhospitable conditions (i.e. no flow and very small enclosures) while in captivity, despite the fact that these species, like *H. comalensis*, only naturally occur in highly oxygenated, flowing waters. Like most aquatic Elmids, adult *S. crenata*, which is described as surviving between 394-398 days in a corked glass vial with no food or water changes (Brown 1974), obtains oxygen via plastron (Thorpe and Crisp 1949).

During this literature review, there were no studies found about prior experiments where flow rates were used to predict invertebrate survival, particularly in subterranean habitats. This study, therefore, will use a novel experimental design to create "spring upwelling" mesocosms in an attempt to shed light on *H.*

comalensis survivorship inside of the springs during periods of low flow and flow cessation. Vertical flow regimes will mimic periods of drought that have caused Comal Springs discharge to decrease to the point that spring upwellings no longer connect the subterranean and surface habitats that *H. comalensis* are likely inhabiting, and will provide information as to how the physical (i.e. temperature) and chemical properties (i.e. DO, pH, conductivity) of spring water change as flow velocity decreases and how beetle survivorship is related to these properties.

Methods

Phase 1: March-April 2014

Phase one of the experimental design includes the preliminary testing of various "substrates" and types of containers to be used as the artificial spring upwelling. The goal of this phase is to determine a substrate, either mesh or clear marbles (Figure 1), etc. that will represent, as closely as possible, the structure of substrate that the beetles would encounter as they traveled down a spring upwelling. The purpose of using a clear substrate is to allow observers to count and to determine the distribution of the beetles within each upwelling throughout the experiment without having to completely take apart each mesocosm, which would likely impact the beetle's behavior during subsequent study. For these experiments we will utilize *Microcylloepus pusillus* as a surrogate species, which inhabit the same areas of Comal Springs as *H. comalensis* and will be collected at Spring Island in New Braunfels, Texas (Figure 2). Upon completion of Phase 1, specifically designed mesocosms will be constructed within the living streams at the Freeman Aquatic Building at Texas State University.

Figure 1. Prototype of the test chamber

Figure 2. Collecting the surrogate species, *Microcylloepus pusillus*, from Spring Island, Comal County, Texas. March 2014.

Phase 2: May-June 2014

Following the preliminary decisions about the construction of the mesocosms in Phase 1, we will begin testing the effect of flow and food availability on *H. comalensis* adults using a factorial experimental design. Each factor (flow and food availability) will have two levels (Yes or No) and the combinations will be tested as follows (Figure 3):

Experimental Condition Number	Factors	
	Flow	Food Availability
1	Yes	Yes
2	Yes	No
3	No	Yes
4	No	No

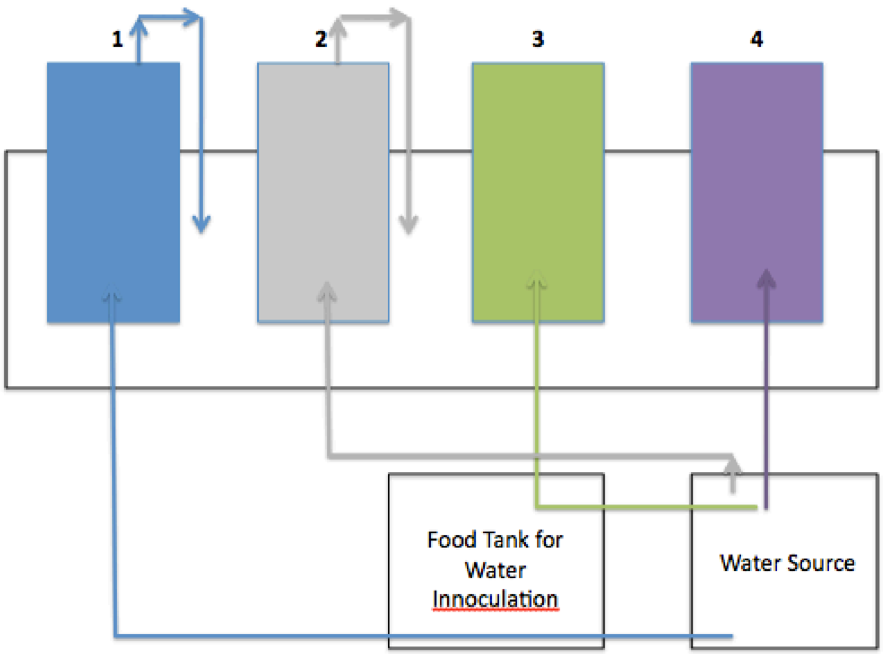

Figure 3. Experimental design and diagram of test chamber layout.

For the parameter of flow, YES relates to flow that reaches the surface and discharges into the spring runs (1 and 2 in Figure 3), while NO is flow from the aquifer that remains subsurface in the test chambers (3 and 4 in Figure 3). Groups of 10 *H. comalensis* individuals will be assigned at random to each experimental condition. This phase will be repeated 3 times (concurrent with one another), which will require 120 *H. comalensis* individuals. All beetles in each treatment will be subjected to the same flow velocity prior to the start of the experiment, which will represent "normal" flowing water. Each round of Phase 2 will last 30 days, with 48 hours of habituation prior to the start of the experiment, during which the no flow treatments will have flows reduced incrementally until flow has been stopped. By utilizing a factorial design, we are able to test each level of the two factors twice per round, which will result in 6 replications of each level by the end of Phase 2. It should be noted that the number of beetles is subject to change based on the number of *H. comalensis* able to be obtained and permitted for the study. Thermisters will be placed in each treatment and set to collect water temperature data every

15 minutes. Water quality parameters (dissolved oxygen, conductivity, and pH) will be tested every 24 hours throughout the experiment. The data collected during Phase 2 will be analyzed using a factorial ANOVA, which will test the independent and interactive effects of flow and food availability in each treatment.

Phase 3: July-August

Phase 3 is dependent on the results of the Phase 2 experiments, thus we have determined 3 options for the experiment that will follow up the initial study.

A) If the results of Phase 2 suggest that *H. comalensis* will survive in both flow and no flow conditions, Phase 3 will explore the duration of time in which the beetles can survive in no flow conditions.
B) If the results of Phase 2 suggest that *H. comalensis* will survive only if food is available to them, Phase 3 will explore whether food from the surface habitats or food from the subterranean habitats is more important for beetle survival.
C) If flow is determined to be a significant effect on adult beetle survival, we will repeat the experimental design from Phase 2 on larval *H. comalensis* to determine if they are as sensitive to flow conditions as their adult counterparts.

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