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LITERATURE REVIEW AND PROPOSED METHODOLOGY FOR STUDY TO ESTABLISH COMAL SPRINGS RIFFLE BEETLE BASELINE POPULATION DISTRIBUTION AND REFINE RIFFLE BEETLE COLLECTION METHODS


Comal Springs riffle beetle collected on a cloth lure.

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Abstract

The Edwards Aquifer Habitat Conservation Plan (HCP) supports applied research for covered species and systems within the plan area. One of the covered species, the Comal Springs riffle beetle (CSRB), *Heterelmis comalensis*, inhabits the Comal Springs system, including Landa Lake, which is fed from springflow issuing from the Edwards Aquifer. Many of the 425 springs or spring groups in the Comal system (Norris and Gibson 2013) were sampled before the springs were mapped and lacked a naming system or coordinated way of tracking which springs were sampled (Norris pers. com. 2014). Over the period of some years Mr. Randy Gibson (USFWS, San Marcos Aquatic Resources Center [SMARC]) has informally tested and refined capture methods for CSRB, with the cultured cotton lure being the most successful method for use in the major spring runs. The purpose of this report is to document the early stages (literature review and study design) of a new project designed to refine collecting methods and map the distribution of CSRB.

The literature review herein is in support of the study design, and focuses on gathering existing biological data and integrating precise geographical information on spring upwellings. Biological information gathered includes the identification of an appropriate surrogate species for lab trials (*Heterelmis glabra*), the examination of a large number of collection methods and choice of three most efficient methods (cultured cotton lures, cultured cotton lures with glow sticks, cultured hemp lures), and the specification of parameters known to support the animals. Geographical literature gathered includes the precise locations of occupied habitat, the GIS files for the Norris and Gibson (2013) mapped springs, and the distribution of survey effort to date.

The laboratory portion of this study will involve using 200 CSRB to test the effectiveness of three lure types (treatments). We will test the treatments in three experimental chambers and have a fourth chamber set up as a control. We will randomly assign each treatment to an experimental chamber, and run three replicates of each treatment over the course of the lab study. Results will be analyzed using an analysis of variance, which will be used to inform the field portion of the study. The lure type shown to be most effective during the laboratory portion of the study will be utilized during the distribution portion of the study.

In order to more clearly define the distribution of CSRB within the Comal system, we will use pre-defined habitat parameters to select springs that have not previously been identified as containing CSRB. Those orifices will be baited for a period of three surveys, or until CSRB are detected. We will utilize an adaptive sampling design to investigate the maximum number of spring orifices possible over the course of this project.

Section 1: Literature Review

As partial fulfillment of contract No. 13-622-HCP, this Literature Review identifies known information related to:

- Location(s) of spring orifices in Landa Lake;
- Currently understood distribution of CSRB in Landa Lake;
- Identification of springs meeting habitat criteria for CSRB; and
- Collection methods (past, present, and those used for surrogate species).

Location(s) of Spring Orifices in Landa Lake

Norris and Gibson (2013) mapped 176 springs in Landa Lake, 142 along the west shore, 92 in spring runs 1 – 3, seven in spring runs 4 – 6, five in the old channel, and three in the spring-fed pool. We obtained the GIS files directly from the authors, and these will be available online for public download in the future.

Currently Understood Distribution of CSRB in Landa Lake

Comal springs riffle beetles have been collected from spring runs 1 – 3 and from the lake at various points along the western shoreline and adjacent to Spring Island. The last discernible attempt at identifying CSRB distribution was made by BIO-WEST in 2001 and 2002 (BIO-WEST 2002a), well before the springs were identified and named by Norris and Gibson (2013). Table 1 provides a list of documented locations of CSRB within the Comal system.

Table 1. General distribution of CSRB based on available literature.

Documented CSRB Locality	Reference
Spring Run 1	cited as Bowles and Stanford 1994 [in USFWS 2013b][1]; Bowles et al. (2000)
Spring Run 2	Bosse (1988); cited as Bowles and Stanford 1994 [in USFWS 2013b]; Bowles et al. (2000)
Spring Run 3	Arsuffi (1993); cited as Bowles and Stanford 1994 [in USFWS 2013b]; Bowles et al. (2000)
Off northeastern tip of Spring Island	BIO-WEST (2002a)
Western shoreline	BIO-WEST (2002a)
Between western shoreline and northernmost peninsula of Pecan Island	BIO-WEST (2002a)

Over the years, Comal Springs riffle beetle sampling has been conducted by quadrats, drift netting, and cotton lures (BIO-WEST 2013a). In the early 1990's extensive sampling in the main Comal spring runs was conducted by Dr. David Bowles (Bowles et al. 2003). Additionally, Mr. Randy Gibson (USFWS ARC) has collected Comal invertebrates at locations throughout the system for a number of projects and for refugia purposes over time. Finally, the EAA biological monitoring program has routinely sampled for the Comal Springs riffle beetle within representative reaches in the Comal system (BIO-WEST 2013b). Figure 1 shows the occupied habitat for the Comal Springs riffle beetle throughout the Comal System as determined for the EARIP Incidental Take Permit Annual Report (BIO-WEST 2013a). That assessment states, "It is anticipated that larger areas of the Comal system are actually occupied than represented in this assessment as the entire Comal system has not been thoroughly sampled. As part of one contracted 2014 HCP applied research study, the distribution and occurrence of the Comal Spring riffle beetle throughout the Comal system will be examined in more detail. It is noted that only surface habitat area was calculated, as the extent of subsurface habitat utilization by this species is presently unknown" (BIO-WEST 2013a).

[1] The primary literature "Bowles and Stanford 1994" was not located during our review.

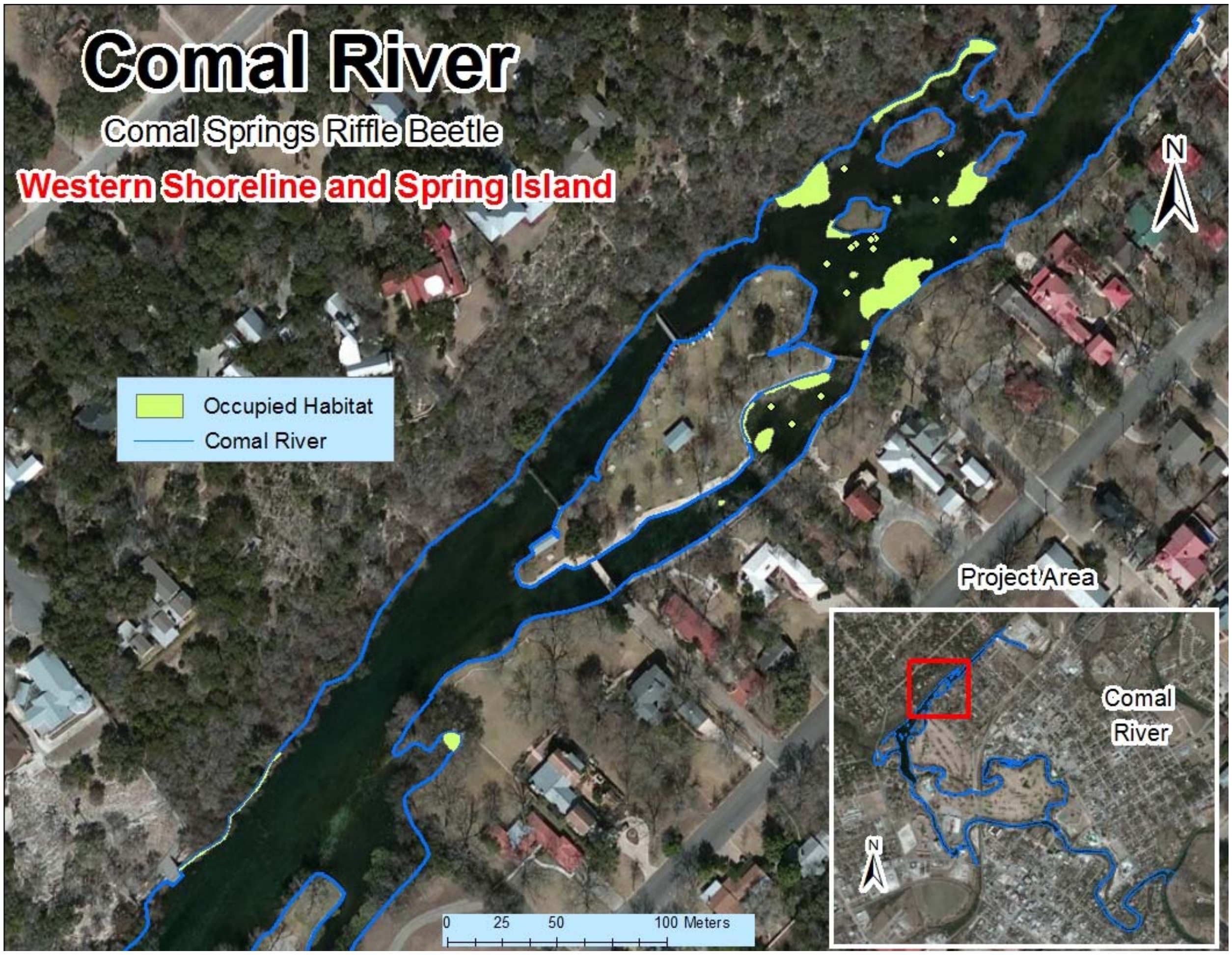

Figure 1. Comal Springs Riffle Beetle Occupied Surface Habitat – Spring Island and Western Shoreline areas (Comal System) (BIO-WEST 2013a).

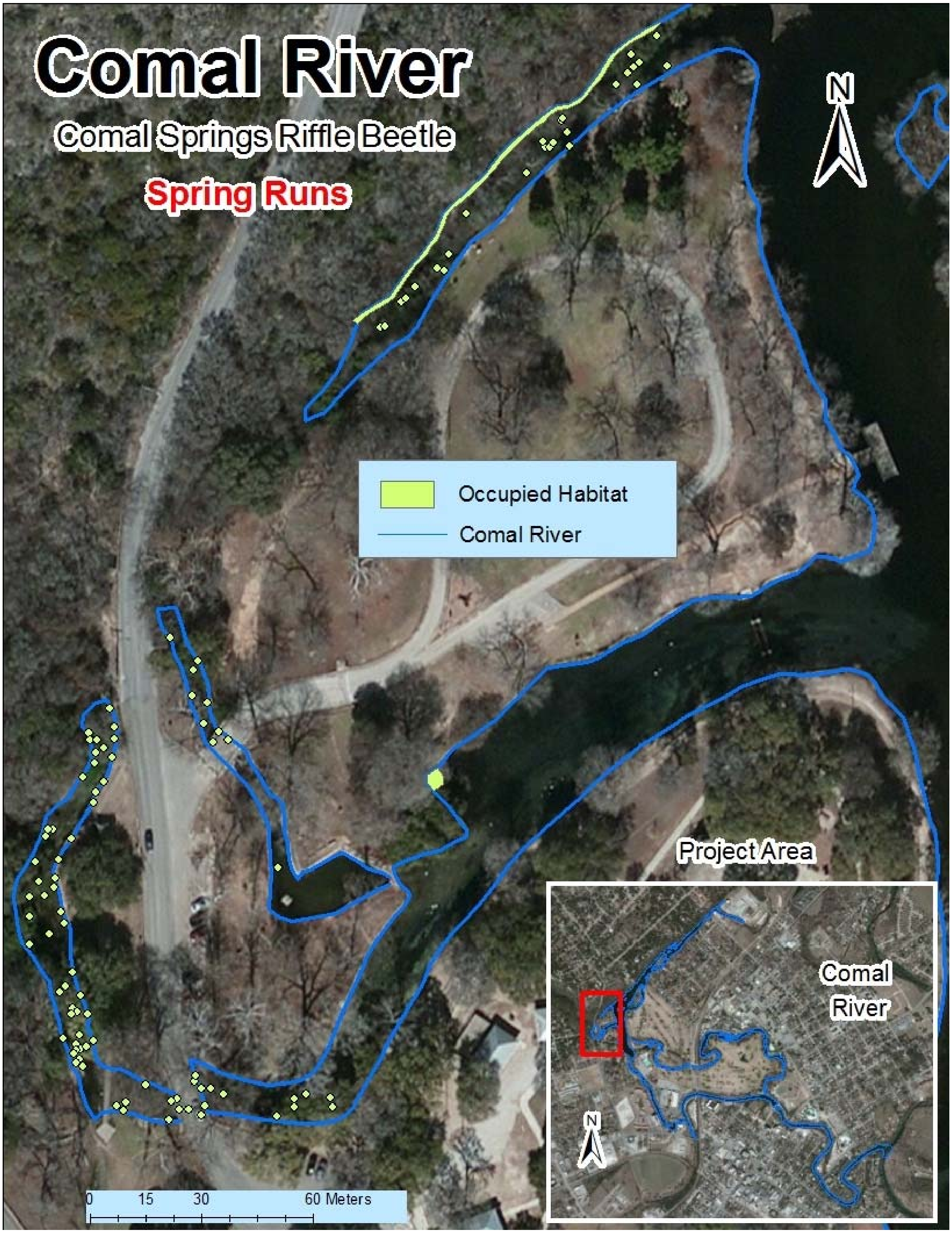

Figure 1 (continued). Comal Springs Riffle Beetle Occupied Surface Habitat – Spring Runs (Comal System) (BIO-WEST 2013a).

Identification of Springs Meeting Habitat Criteria for CSRB

Bowles et al. (2003) identified CSRB habitat as having fast flowing water with gravel and cobble substrate, and BIO-WEST (2002a) documented that they are not detected in silt covered areas. Laboratory studies have shown that CSRB prefer spring outlet conditions and water quality parameters typical of Edwards Aquifer water, and that they tend to distribute themselves closer to well water than purified water in preference studies (Cooke 2012).

The USFWS (2013a) defined the following primary constituent elements of riffle beetle habitat:

(1) Springs, associated streams, and underground spaces immediately inside of or adjacent to springs, seeps, and upwellings that include:
    (a) High-quality water with no or minimal pollutant levels of soaps, detergents, heavy metals, pesticides, fertilizer nutrients, petroleum hydrocarbons, and semi-volatile compounds such as industrial cleaning agents; and
    (b) Hydrologic regimes similar to the historical pattern of the specific sites must be present, with continuous surface flow from the spring sites and in the subterranean aquifer.

(2) Spring system water temperatures that range from 68 to 75°F (20 to 23.4°C).

(3) Food supply that includes, but is not limited to, detritus (decomposed materials), leaf litter, living plant material, algae, fungi, bacteria, other microorganisms, and decaying roots.

For the purposes of identifying springs to sample within the framework of water quality and physical parameters measured in Norris and Gibson (2013), we define CSRB habitat based on the following parameters:

- Spring type: upwelling
- Temperature: between 68° – 75° F,
- Flow sufficient to maintain clear substrate conditions in the immediate spring orifice, and
- Substrate consisting of organics, sand, coarse sand, very small gravel, small gravel, medium gravel, large gravel, rubble, small cobble, large cobble.

Collection Methods

Several collection methods have been employed on CSRB over the years. BIO-WEST (2002a) reports using manual collection methods utilizing fingers and/or soft forceps and dip-nets. More recently, Gibson et al. (2008), BIO-WEST (2013b) and Gibson (pers. comm. 2014) reported success with cotton cloth lures allowed to "culture" in situ for a period of four weeks. At approximately four weeks, the cotton lures grow fungus believed to attract CSRB.

Abandoned collection methods include the use of cotton lures inside PVC tubing, which CSRB appear not to enter (Gibson 2014 pers. comm.), and sampling with a surber, which was considered in BIO-WEST (2002a) but not employed due to lack of adequate flow. Drift net sampling immediately over spring orifices (BIO-WEST 2013b) has also proven to be ineffective for collecting more than a few individuals per sampling event.

Collection methods previously used on aquatic macroinvertebrates that have not been attempted for CSRB collection include bottle traps (Needham 1924, Kellen 1953, Husbands 1960), lighted bottle traps (Husbands 1967, Washino and Hokama 1968, Carlson 1971, Espinosa and Clark 1972, Apperson and Yows 1976, Aiken 1979), substrate screening, artificial leaf packs (Petersen and Cummins 1974, Benefield et al. 1977, King 1987, Hieber and Dobson 1991, Gessner 1991, Dobson and Hildrew 1992, Richardson 1992, Dobson 1994, Murphy et al. 1998, and Gessner 2002) and Berlese funnel (Hilsenhoff and Tracy 1985). A Berlese funnel is less desirable because it would result in direct take of every individual in the sample, however it could be beneficial on a small scale in order to determine CSRB use of substrate along the shore. Another lethal method described in Hilsenhoff and Tracy (1985) involves a quickly collected sample using a metal cylinder treated with pyrethrin, which causes beetles to rise to the surface of the collected water for collection. Each of these novel methods has problems that we discussed with other researchers, including lethality, difficulty of sorting, and lack of use by CSRB for similar trap types.

Section 2: Proposed Methodology

Study objectives

Comal Springs riffle beetle distribution throughout Landa Lake and the Comal system is currently unknown. Collection methods have been somewhat effective; however, the various methods have not been tested in a controlled setting or documented scientifically. The methods could potentially be improved. The purpose of this study is to test the success rate of three collection methods on CSRB and to establish a distribution of spring orifices that are occupied riffle beetle habitat.

Beetle collection and housing

We will perform preliminary trials with a surrogate species, the riffle beetle *Heterelmis glabra* (no common name) during development of the experimental systems. Specimens of *H. glabra* will be collected from the Devils River in west Texas. This species is the closest known relative of the CSRB (Brown and Barr 1988). We will test two refugia using the surrogate species. After preliminary testing, we will leave the beetles in one refugium for future preliminary trials associated with EAHCP Applied Research projects.

We will collect a maximum of 250 CSRB from spring runs 1-3. Beetles will be carefully removed by hand or soft forceps and placed in a small cooler containing spring water and a few leaves collected on site. After transport to the Freeman Aquatic Building, Texas State University - San Marcos, we will confirm identification of the beetles in the laboratory with the aid of a dissecting microscope. After confirmation, we will move the beetles to the unoccupied refugium.

We will store collected beetles in aquaria, keeping the two riffle beetle species separate. We will employ heater units (if necessary) to maintain water temperature between 21-23 C (range recorded in natural habitat, Brune 1981) and a pump to circulate water throughout the tank. We will only use untreated water pumped directly from the Edwards Aquifer in each tank. Anacua (*Ehretia anacua*) leaves collected from areas around the Comal River will be dried and introduced into an aquarium filled with aquifer water to serve as a source of bacterial and fungal growth. We will circulate water through the leaves, filter the water, and add it back into the holding tank to evenly inoculate areas of each holding tank with epiphyton on which the beetles might feed.

For this study, we will utilize approximately 150-200 of the collected beetles. The remainder of the beetles (~50-100) will be used in BIO-WEST's 2014 HCP applied research study evaluating the effects of low-flow. All surviving beetles from both studies will be transported to the USFWS ARC for use in refugia under the guidance of Randy Gibson.

Experimental Design

We will utilize four experimental chambers for the laboratory portion of this project. Untreated Edwards Aquifer water will be pumped directly into the chambers to maintain the proper temperature, dissolved oxygen (DO), and pH. A pump will be utilized to create flow.

We will carefully remove approximately 50 CSRB from refugia with disposable plastic transfer pipettes and place them in plastic beakers partially filled with well water. We will then pour the water and beetles into the center of the first experimental chamber. We will repeat this procedure three times until there are the same number of CSRB in each chamber (approximately 50). We will then assign three of the chambers as test systems and the forth will serve as a control.

Treatments we propose testing include the standard cotton lure, cotton lure with glow sticks attached, and a hemp lure. The standard cotton lure is the most effective CSRB collection method realized to date (Gibson 2008, Gibson pers. comm. 2014). This method involves placing a strip of cotton cloth inside of a mesh wire container and submersing it in spring orifices for 3-5 weeks to allow microorganisms (primarily fungi) to grow on the cotton cloth (Figure 2).

Figure 2. Cultured cotton lure.

We chose to include the cloth lure with glow sticks in our study because lighted funnel bottles have been successfully used to collect other aquatic macroinvertebrates (Walker 1955, Husbands 1967, Washino and Hokama 1968, Carlson 1971, Espinosa and Clark 1972, Apperson and Yows 1976, Aiken 1979) and other species of adult riffle beetles are sometimes attracted to bright lights placed near streams (Evans and Hogue 2006). This method will be identical to the standard cotton lure method, but will have two glow sticks attached to the wire frame. The hemp lure method will be identical to the standard cloth lure; however, we will use a strip of hemp cloth instead of cotton. This method has not been tested but the material, like cotton, would resemble the breakdown of organic debris such as roots and leaves. We will use the wire frame without a lure in the control.

We will test each method by placing the different lures in each chamber and running the treatments simultaneously for a period of 24 hours. After the treatment is complete, we will remove all lures and place them in separate trays containing well water. We will tally the number of CSRB obtained using each treatment as well as the number of beetles remaining in the chamber. We will then carefully remove all collected beetles from the traps with disposable plastic transfer pipettes and place them in beakers partially filled with well water. After counting, we will pour the water and beetles back into the same chamber from which they were collected. After a 24-hour rest period, we will rotate treatments between the chambers. Each treatment will be replicated three times.

We expect these results:

-percentage of individuals captured in each lure during the 24 hour test periods

-comparison of percentage captured across 4 tanks and 4 variables (3 types of lures and control) using an ANOVA

All CSRB used in this portion of the study, along with the surrogate specimens, will immediately be made available to other EAA researchers.

Population Distribution Study

We will use an adaptive sampling method to test for presence in springs where CSRB have not been confirmed. Cultured lures will be placed at spring outlets likely to have appropriate CSRB habitat based on the above-defined parameters, and will be checked not more frequently than twice per week over a period of four to eight weeks. We will maintain a list of springs appearing to contain appropriate habitat, and will begin sampling at springs that have the highest likelihood of containing CSRB, based on known and assumed habitat parameters and on coordination with other species' experts. Springs with positive detection will not be re-trapped and will be noted and removed from the list to be replaced with the spring with the next highest likelihood of CSRB that is not already being trapped. We will survey for CSRB up to three times in each of the springs before moving down the list. In order to sample the maximum number of springs possible within the time and budgetary constraints of the project, we will not make any attempt to gather additional habitat data other than our initial spring selection criteria.

We expect these results:

-an updated map of CSRB distribution presence/absence

-a comparison of numbers of animals per trap across different spring orifices

-an examination of other parameters that may contribute to numbers of animals per trap

-a discussion of the approximate number of sampling events that typically leads to determining presence or absence

-a discussion on the nature of the changing spring locations in relation to presence/absence studies

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