COMAL SPRINGS RIFFLE BEETLE OCCUPANCY MODELING AND POPULATION ESTIMATE WITHIN THE COMAL SPRINGS SYSTEM, NEW BRAUNFELS, TEXAS

Spring Habitat of the Comal Springs System

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Abstract

We sampled for the federally-listed *Heterelmis comalensis* (Comal Spring riffle beetle) at 95 randomly chosen spring outlets in the Comal Springs complex. Field biologists with expertise in the identification of aquatic macroinvertebrates sampled each site three times, with a resting period between sample events. During each sample event, technicians measured a series of eight covariates thought to impact occupancy or detection. The sampling occurred during a period of extreme low flow in October 2014, and resulted in detecting a total of 137 Comal Spring riffle beetles. Using the program PRESENCE, the highest ranking model indicated that the covariates 'spring type,' or specifically spring orifices, and 'presence of roots and/or detritus' were possible predictors of occupancy; however, seven additional models were also supported.

Using the repeated count data and an N-mixture abundance model, we extrapolated our point counts to the total amount of available habitat to calculate an estimate of 741 beetles in the system (90% CI 471-1,284) at that time. This estimate is drastically different from existing estimates that use extrapolation over wetted area. Because population estimates derived from surveying species within a subset of their habitat without using mark-recapture techniques can be problematic, the population estimates provided from our study should be interpreted cautiously.

Introduction

Heterelmis comalensis (Comal Spring riffle beetle) occurs in springs and seeps issuing from the Edwards Aquifer in the Comal River and Landa Lake (Comal Springs system), Hueco Springs, Fern Bank Springs, and in the upper reaches of San Marcos Springs (Spring Lake), Comal and Hays Counties, Texas (Gibson et al. 2008). *H. comalensis* was listed as an endangered species by the U.S. Fish and Wildlife Service (USFWS) in 1997, primarily due to factors threatening the flow of spring water at Comal and San Marcos Springs (USFWS 1997). *H. comalensis* is usually found where spring flow is evident in areas with rock and gravel substrates that are free of silt (BIO-WEST 2002).

As part of the Edwards Aquifer Authority's ongoing Variable Flow Study, BIO-WEST, Inc. (BIO-WEST) has conducted semi-annual and critical period monitoring of *H. comalensis* since 2002 to gather information on population dynamics under varying flow conditions. Critical period monitoring occurs when established high and low flow trigger levels are reached. When the study began, *H. comalensis* was only known at Comal Springs from spring runs 1, 2, and 3. In 2002, their known range within the Comal Springs system expanded when BIO-WEST found them in upwelling areas of Landa Lake and Spring Island (BIO-WEST 2002).

In 2013, the Edwards Aquifer Authority, San Antonio Water System, City of San Marcos, City of New Braunfels, and Texas State University were granted an Incidental Take Permit (ITP) allowing incidental take of threatened and endangered species from activities involving regulating and pumping of groundwater from the San Antonio segment of the Edwards Aquifer, and the recreational and commercial use of Comal and San Marcos spring and river systems. The Habitat Conservation Plan developed to support issuance of the ITP includes a two-phased approach to minimize and mitigate take, and to ensure that the covered activities will not appreciably reduce the likelihood of survival or recovery of those species. Phase I involves implementation of minimization and mitigation measures to provide protection. An Adaptive Management Process will use information gathered from improved biological and ecological models to make appropriate modifications to the program. This process is designed to track progress towards the long-term goals of the plan, and to learn more about the cause-and-effect relationships responsible for the variability in the habitat and population measures.

The goals of the Ecological Model for *H. comalensis* are "to evaluate potential adverse ecological effects from Covered Activities, and to the extent that such effects are determined to occur, quantify their magnitude" and "to develop alternative approaches or possible mitigation strategies, if necessary" (Recon et al. 2012). In order to improve the biological and ecological model for *H. comalensis*, a population estimate is needed to establish a general baseline for monitoring. The objectives of this study are to determine the level of occupancy for *H. comalensis* within the Comal system and obtain a system-wide estimate of population size to help inform the Ecological Model.

Methods

Study Area

Our study area encompassed the Comal Springs system in New Braunfels, Comal County, Texas (Figure 1). The Comal Springs system is the largest spring system in Texas (Brune 1981). The high-quality groundwater issuing from the springs is fed by the Comal Springs fault of the eastern part of the Edwards Aquifer (LBG-Guyton and Associates 2004). Most of the springs are actually spring complexes, or groups of springs with multiple outlets or areas of diffuse flow. In 2012, 425 springs were mapped in the Comal system and assigned to the following areas: spring runs 1 through 6, the western shoreline, the bottom of Landa Lake, Spring Island, and the old channel.

Sampling Design

To best meet our objective of providing an overall level of occupancy of *H. comalensis* within the Comal Springs system, we chose to randomly select survey sites throughout the entire system rather than stratify according to location or habitat type. Stratifying according to location or habitat type is not considered optimal if the primary objective of the study is to determine a system-wide level of occupancy (MacKenzie and Royle 2005). Biased population estimates may result when site selection is based on pre-existing knowledge about their potential state of occupancy (MacKenzie and Royle 2005). Our sample frame, from which sample units were selected, consisted of all spring outlets within the system.

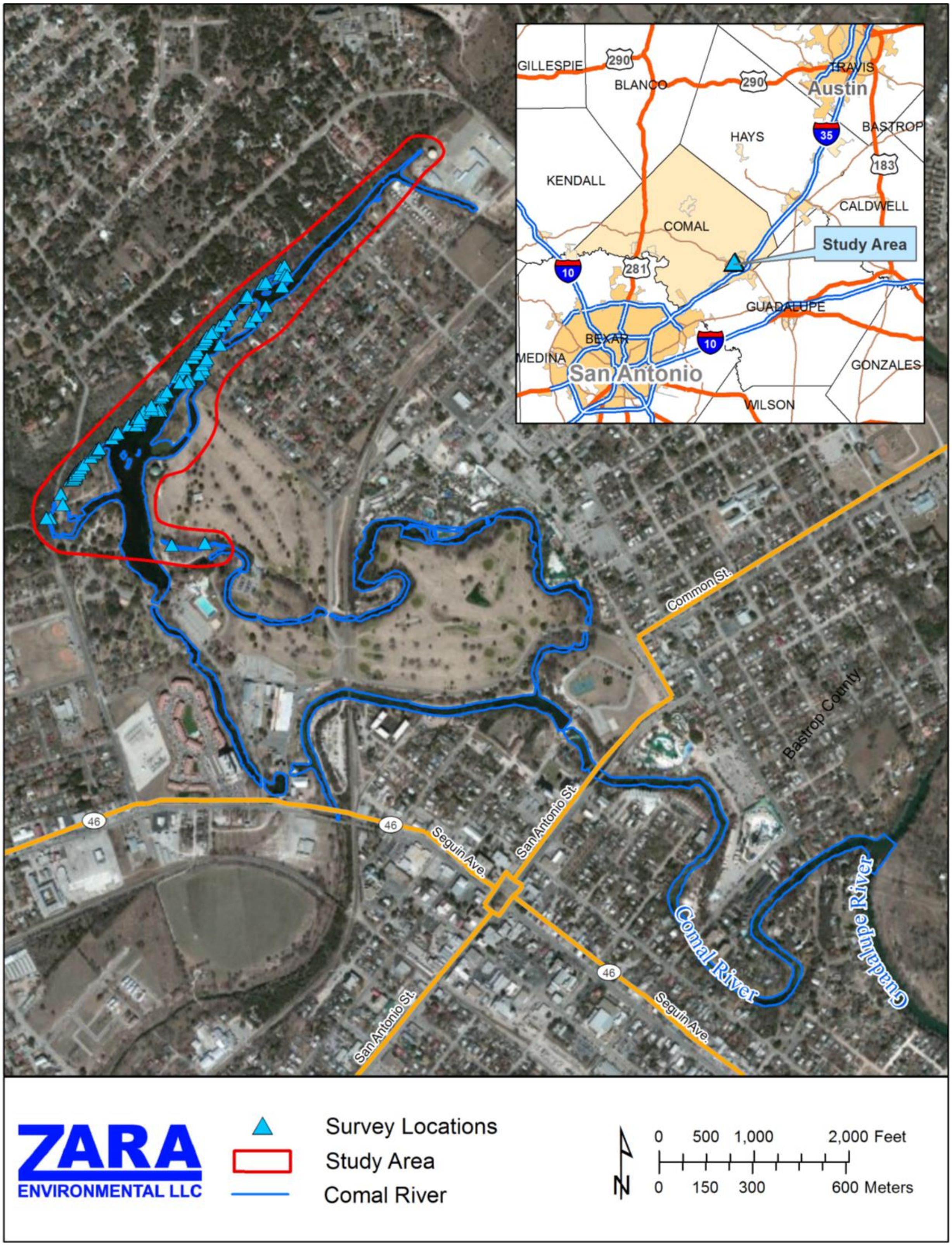

Figure 1. Comal Springs riffle beetle occupancy model and population estimate study area.

We defined spring outlets as any area with visibly detectable spring flow. While examining the study area for potential sample locations, we focused on locations that Norris and Gibson (2013) mapped as springs during normal flow conditions. However, during our study period, the system was experiencing extreme low flow conditions and many of the springs previously mapped were dry or reduced to a seep. When it was difficult to identify a spring due to low or diffuse flow, we focused on areas that were both previously mapped as springs and that had little or no fine sediment accumulation, and/or areas producing bubbles. We confirmed flow in these areas by close observation, using a SCUBA mask, of movement of fine substrate matter.

We chose a minimum distance of two meters between sites to ensure independence among sample sites. Cooke (2012) observed that CSRB abundance was highest within 20 cm of spring outlets and no CSRB were found greater than 80 cm away from a spring outlet. We applied this assumption to other researchers' lures as well. BIO-WEST was conducting low-flow monitoring of *H. comalensis* while we selected our sites. If our random number generator picked one of their survey sites, we selected the next available site that was at least 2 meters away.

Given our two-meter minimum sample distance, we mapped every possible sample site in the system during this time and found approximately 300 sites available for sampling. We used a random number generator in the field to select one third of those available sites, with a total number of 95 sites selected. Once selected, each site was given a number and coordinates were recorded with a Trimble GeoXH6000. We marked each site in the field using a combination of flagging, colored rocks, and rock cairns (Figure 2).

Figure 2. Rock cairn and flagging marking a survey site.

Surveys

We conducted three sampling events at our randomly chosen sites in October of 2014. We used a passive sampling technique using cotton-cloth lures to survey for *H. comalensis*. This method is proven to be highly effective and is recommended for sampling this species (Gibson et al. 2008). We deviated somewhat from this technique by culturing our lures prior to each sampling event in an area where detections of *H. comalensis* during monitoring events are common. Culturing the lures is a process where field staff bury the cotton cloth in a spring orifice to grow a light covering of natural mold and bacteria that likely serves as a food source for the beetle (Gibson et al. 2008). We cultured lures in advance at a single location in order to normalize the treatment of the lures, and to avoid potential bias based on differing lure cultures. Various spring orifices are known to be fed from different groundwater sources (LBG-Guyton and Associates 2004) and may provide different mold and bacteria colonies, potentially resulting in variation of the attractiveness of our lures.

To culture our lures, we placed strips of cotton-polyester blend cloth into 120-micron mesh bags and sealed them with multiple folds and stitches. We placed the bags into a spring outlet along the western shoreline where *H. comalensis* is known to occur. The mesh bags were retrieved after approximately three weeks, and invertebrates and their larvae were removed from the outside of the bags before removing them from the system. We removed the cultured strips of cloth from the mesh bags and carefully viewed them under a dissecting microscope while in the field, removing any larvae that may have been small enough to fit through the 100-micron mesh fabric. This would only apply to *H. comalensis* larvae of the smallest known instar with a diameter of 0.13 mm (Cooke 2012). We cut the cultured strips of cloth into 30 cm squares and placed them in wire mesh cages marked with the site numbers (Figure 3). We attempted to cut lures so that each one had approximately the same amount of cultured material growing on it. We buried one lure in the substrate of spring outlets at each of our chosen sites. Lures were placed close to or, if possible, inside the spring source and buried approximately 15 - 30 cm into the substrate (Figure 4).

After five days, we retrieved the lures and removed *H. comalensis* adults and larvae to confirm their identification in the field using a dissecting microscope (Figure 5). We recorded the presence or absence of *H. comalensis*, counted *H. comalensis* adults and larvae, recorded any other notable invertebrates on the lure, and measured and recorded each of the covariates discussed below. After recording our data, we returned the animals to the system in the same location. As a rest period, we separated each sampling event with 72-hours where no lures were present in the system. This rest period allowed any recently trapped or attracted animals to return to their natural habitat, and allowed us to consider the next trapping event independent from the previous one.

Figure 3. Cultured cotton lure next to wire mesh cage.

Figure 4, Surveyor burying lure in wire cage into substrate.

Figure 5. Researchers examine beetle larvae under a dissecting microscope.

The same researchers performed each sampling event to reduce potential observer bias. Researchers identifying beetles were experienced in conducting *H. comalensis* surveys, and experienced BIO-WEST researchers assisted us in the field during the first survey to aid in identification of beetles and larvae. Particular attention was paid to the minor nuances of differences between *H. comalensis*, *Microcylleopus pusillus*, and *Stygoparnus comalensis* (also federally-listed) species, both as adults and instars.

Occupancy Model

We used a single-season occupancy model approach to estimate *H. comalensis* probabilities of occupancy and detection across the study area, given certain environmental parameters (MacKenzie et al. 2002). This approach allowed us to test different biologically relevant hypotheses (i.e. models) and determine which model best fits the data based on Akaike's Information Criterion (AIC) (Akaike 1973). AIC provides a reliable decision criterion for model selection for both nested and non-nested models (Schmidt and Anholt 1999, Burnham and Anderson 2002).

We selected a suite of a priori hypotheses regarding factors that could influence *H. comalensis* occupancy and detection, both positive (+) and negative (-), within the Comal Springs system (Table 1). We hypothesized that shade, substrate, the presence of roots and/or detritus, and siltation could influence occupancy of *H. comalensis* because they are usually observed in shaded spring outlets with gravel substrates between 8-128 mm that have roots and/or detritus present, and are free of excessive siltation (BIO-WEST 2002, Bowles et al. 2003, Ed Oborny, BIO-WEST, Inc. personal communication).

Table 1. Hypothesized positive (+) and negative (-) relationships between covariates and *H. comalensis* probability of occupancy (Ψ) and detection (p).

	Ψ (Occupancy)	ρ (detection)
Spring Type	+/-	+/-
Location Spring Runs 1-3	-	-
Flow	+/-	+/-
Recent Rain		-
Shade	+	
Substrate size 8-128 mm	+	
Detritus or roots present	+	
Silt	-	

We also hypothesized that spring location and spring type may influence occupancy during times of drought. Because springs in spring runs 1 through 3 dry faster and for longer periods of time during extreme low flow events, local extinctions are more likely to occur in this area under drying conditions, reducing occupied areas to upwellings in and around Spring Island and Landa Lake proper (LBG-Guyton and Associates 2004, Gonzales 2008). We also suspected that beetles would be less likely to occupy orifices because a single orifice is more likely to have higher velocities and provide fewer interstitial spaces in the form of loose gravel for the beetle to use as cover. Cooke (2012) found that CSRB prefer to be in low flow and avoid areas of high flow as well as light. Additionally, researchers have documented that CSRB are most often found in interstitial spaces, such as under gravel where flow is lower, presumably to prevent from being swept downstream (Cooke 2012).

We suspected that spring location, flow, and recent rain might also influence detection. Because *H. comalensis* are thought to retreat deeper into the substrate and possibly reduce foraging activities with dropping water levels and changing water chemistry, they may not be as likely to find the lure (Ed Oborny, BIO-WEST, personal communication). As stated previously, this is most likely to occur first and last the longest in spring runs 1 through 3 (LBG-Guyton and Associates 2004). We further hypothesized that spring type may negatively impact detection. If the morphology of the spring outlet does not allow for placement of the lure directly over or next to the outlet, it may skew the ability for the beetles to find the lure. As mentioned previously, *H. comalensis* abundance was highest within 20 cm of a spring outlet and they were not observed in areas greater than 80 cm away (Cooke 2012).

We separated each site by location (0 = areas other than spring runs 1-3, 1 = spring runs 1-3) and spring type (1 = alluvial, 2 = upwelling, 3 = orifice). Shade was recorded as an estimated percentage of shade covering each site over the course of a day (0 = not primarily shaded, 1 -= primarily shaded). We categorized substrate as being dominated by gravel and/or rocks ranging from 8-128 mm diameter (0 = substrate not 8-128 mm, 1 = substrate 8-128 mm). We recorded whether roots and/or detritus was present at each site (0 = roots/detritus not present, 1 = roots/detritus present). We documented if the substrate was primarily silted over or not (0 = silt not dominate, 1 = silt dominant), and we recorded the observed flow (1 = flow low, 2 = flow medium, 3 = flow high). We did not measure flow with a flow-meter because flow was too low or diffuse at the time we surveyed for reliable readings. We also recorded if there had been a recent significant rain event prior to surveying for beetles (0 = rain < 2.54 cm within previous two days, 1 = rain > 2.54 cm within previous two days).

Models were estimated using the program PRESENCE (version 6.9), with the dependent variable being coded as a binary (0 = no beetles; 1 = at least one beetle). The model with the lowest AIC was considered the best approximating model. Models were ranked based on AIC scores. Point estimates, standard errors, and 95% confidence intervals were recorded for Ψ (occupancy) and *p* (detection) for each model. If the difference in AIC (ΔAIC) between the best fit model and each competing model was < 2.0, then the models were considered to be statistically indistinguishable (Simonoff 2003). Models within 2-7 ΔAIC were also supported (Burnham and Anderson 2004).

Population Estimate

We used an N-mixture repeated count model (Royle 2004) as implemented in the package "unmarked" (Fiske and Chandler 2011) in R (R Development Core Team 2008) with an AIC approach to model selection (incorporating the same covariates as used in occupancy modeling) and assuming a negative binomial distribution to estimate CSRB abundance at our sample sites. To provide a system-wide population estimate, we extrapolated our point counts to the total amount of available habitat as *total = 3 x sampled* area (1/3 of available habitat was sampled).

Results

Surveys

Surveys documented a total of 137 *H. comalensis* observations or detections, including 101 beetles and 36 larvae. The species was detected in 22 out of 95 spring outlets (Figure 6). Because we surveyed 95 sites three times, we had 285 opportunities for detection. We detected *H. comalensis* 38 times, providing us an extended naïve occupancy estimate of 0.13.

In addition to *H. comalensis*, a number of other aquatic invertebrates were observed on our lures during surveying, including the federally-listed endangered *Stygoparnus comalensis* and *Stygobromus pecki*. We found three *S. comalensis* and 78 *S. pecki*. All *S. comalensis* observed were at sites also occupied by *H. comalensis*. We observed *S. pecki* on lures at 29 of our sites, which overlapped with 10 *H. comalensis* detection sites.

Figure 6. *H. comalensis* sample locations and detections within the study area. Note: due to low flows, there were no potential sample locations identified in or around spring runs 4 or 5.

Occupancy Model

We considered the suite of variables provided in Table 1, but preliminary analyses indicated that spring type, location, and roots and/or detritus present should be the only variables used to parameterize the occupancy model due to an uneven distribution of observations for the other variables. The highest ranking model of *H. comalensis* occupancy and detection (detritus, and that detection probability remained unchanged across survey events. Data in Table 2 suggests that occupancy is positively correlated with spring orifices and the presence of roots and/or detritus, and that detection probability remained unchanged across survey events. The next best model, with ∆AIC value of 1.95, shows the same correlations, but also that detection probability changed across survey events. The most parsimonious model that is supported, with ∆AIC of 5.41, is the model with static occupancy and detection probabilities. The most parsimonious model was within seven ∆AIC of the highest-ranking models, thus statistically indistinguishable from the other models presented in Table 2 (Burnham and Anderson 2004). Using this model, we determined that the probability of occupancy was 0.26 (SE 0.05, 95% CI [0.18, 0.38]) and the probability of detection was 0.51 (SE 0.08, 95% CI [0.36, 0.65]).

Table 2. Highest ranking models from a single-season occupancy analysis for *H. comalensis* at Comal Springs in Texas, 2014. Ψ = occupancy, p = detection. We considered models within 7 ∆AIC to have support. AIC = Akaike's Information Criterion. Modeling of a parameter as static is indicated by "(.)".

	AIC	∆AIC	AIC Weights	No. Parameters
Ψ (spring type + roots), p (.)	187.03	0.00	0.421	5
Ψ (spring type + roots), p (variable)	188.98	1.95	0.159	7
Ψ (spring type + location + roots), p (.)	189.03	2.00	0.155	6
Ψ (roots), p (.)	190.13	3.10	0.089	3
Ψ (spring type + location + roots), p (variable)	190.98	3.95	0.058	8
Ψ (location + roots), p (.)	192.13	5.10	0.033	4
Ψ (.), p (.)	192.44	5.41	0.028	2
Ψ (spring type), p (.)	192.54	5.51	0.027	4

Population Estimate

By applying an AIC approach to abundance model selection incorporating the same covariates used in occupancy modeling, the two highest ranked models included spring location and presence of roots as predictors of abundance. Similar to the results of *H. comalensis* occupancy modeling, however, a number of other models were also supported (Table 3). As the most parsimonious model (static detection and abundance estimates) was within two AIC of the highest-ranking models (and is thus indistinguishable statistically), this model was chosen to generate abundance and detection estimates. Detection was estimated as 0.183 (95% CI 0.138 - 0.238). This model produced a point population estimate of 2.63 (95% CI 1.33 - 5.17) beetles per site. The Bayesian empirical best unbiased predictor (EBUP) for *H. comalensis* within the 95 sites we sampled was 247 beetles, with a 90% confidence interval of 157 - 428 beetles. When we extrapolated our point counts to the total amount of available habitat, we produced an estimate of 741 beetles in the system (90% CI 471-1,284) at the time we conducted our surveys.

Table 3. Highest ranking abundance models from repeated count modeling of *H. comalensis* at Comal Springs in Texas, 2014. *N* = abundance, p = detection. We considered models within 7 ∆AIC to have support. AIC = Akaike's Information Criterion. Modeling of a parameter as static is indicated by "(.)".

	AIC	∆AIC	AIC Weights	No. Parameters
N (roots), p (.)	391.07	0.00	0.368	4
N (location + roots), p (.)	392.05	0.98	0.225	5
N (.), p (.)	392.96	1.90	0.142	3
N (spring type + roots), p (.)	393.11	2.05	0.132	6
N (location), p (.)	394.91	3.84	0.054	4
N (location + spring type + roots), p (.)	395.04	3.97	0.050	7
N (spring type), p (.)	396.93	5.87	0.020	5
N (location + spring type), p (.)	398.73	7.66	0.008	6

Discussion

Occupancy Model

Overall, our estimates of occupancy and the distribution of *H. comalensis* are consistent with known occupancy and distribution of the beetle within the study area (BIO-WEST 2002, Bowles et al. 2003). Compared to the other models examined, our highest ranked model suggests that *H. comalensis* occupancy is positively correlated with spring orifices and the presence of roots and/or detritus, and that *H. comalensis* detection is constant across survey sites and occasions. However, as mentioned previously, there are six additional models presented with ∆AIC scores between 2 and 7 that cannot be discounted (Burnham and Anderson 2004). The parameters tested were not informative in this case, likely because there is insufficient information currently available regarding the species' life history and distribution to properly parameterize an occupancy model. However, because the covariate 'spring type' was found in five of the eight models supported and 'presence of roots and/or detritus' is found in six of the eight models supported, those variables may affect *H. comalensis* occupancy and should not be ruled out for inclusion in future occupancy studies.

Possible relationships with *H. comalensis* occupancy or detection may have been missed because the study design was not stratified to include sites representing an equal distribution of covariate categories. For example, while 73% of our sites with detections had substrate sizes ranging from 8-128mm, 80% of our sample locations also fit into that substrate category. Thus, we likely did not survey enough sites representing substrate sizes outside of that range to detect any significant affects. Likewise, only six percent of our sites were dominated by silt during the course of our study, resulting in the same problem. Future studies examining environmental factors influencing *H. comalensis* occupancy could use a stratified sampling design to increase the potential of detecting influential habitat characteristics.

Population estimate

Reduced detection, inadequate sampling techniques, or reduced population size?

The drought of the 1950's is the worst on record at Comal Springs (LBG-Guyton Associates 2004, USGS 2015). During that time, water ceased flowing in spring runs 1 through 3 (LBG-Guyton Associates 2004), but the CSRB population in those areas survived. It is hypothesized that the species moves down into the substrate beneath receding water levels during times of drought (USFWS 1997). This hypothesis is supported by research conducted by BIO-WEST (2007), indicating that the beetle prefers to be in and move toward the current and downwards. The low numbers of beetles detected, especially in the spring runs, during our survey may be at least partially a result of non-detection at occupied sites (due to the beetles retreating down into the subsurface making it more difficult for them to find our lures) and/or inadequate sampling techniques. However, our data analysis reveals that we did detect *H. comalensis* 51% of the time in occupied sites.

The low numbers could also be the result of negative impacts on the population due to reduced flow over an extended period of time. USFWS (1997) states that "…a period of extensive, long-term cessation of spring flow likely would not (allow the survival of these species). Because these invertebrates are fully aquatic and require relatively well oxygenated water, a reduction or cessation of spring flows, even if standing water remains around the spring orifices, may negatively impact the species." Genetic analyses on CSRB in 2008 found that the spring runs 1-3 and backwater spring populations are genetically invariant and data from that study supports the hypothesis of "recent and severe bottleneck events" in those areas (Gonzales 2008). The true answer to the question likely depends on the species' survival threshold under the conditions at that time (how long at particular flow rate before the population is negatively impacted), which we do not currently understand.

Our study occurred during one of the worst droughts ever recorded at Comal Springs. Figure 7 displays USGS flow data for the Comal River that includes the worst drought on record during the 1950's with the drought occurring during the time of our survey.

Monitoring data provided by BIO-WEST from surveys performed in 2014 exhibit declines during the peak of the drought, and are consistent with our survey data. This indicates that the low numbers we obtained are not likely due to inadequate sampling techniques during our survey, but rather to the ongoing drought conditions and shrinking surface habitat resulting in either a population decline or reduced detection (or both). In July 2014, when the flow averaged 118 cubic feet per second (cfs), BIO-WEST counted a total of 297 beetles at 30 springs; however, by September 2014 when the average flow was 80 cfs, their total count dropped to 104 beetles (Figure 8).

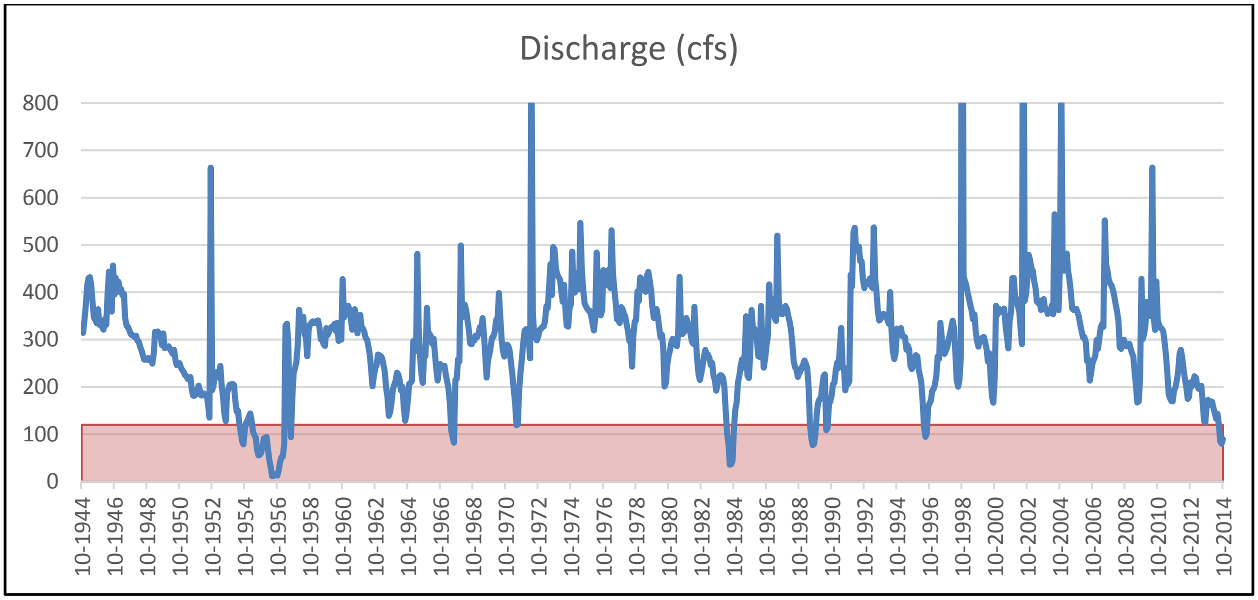

Figure 7. US Geological Survey flow data for the Comal River from October 1944 to November 2014. The shaded area represents flows of 120 cfs or lower, a low flow sampling trigger for BIO-WEST.

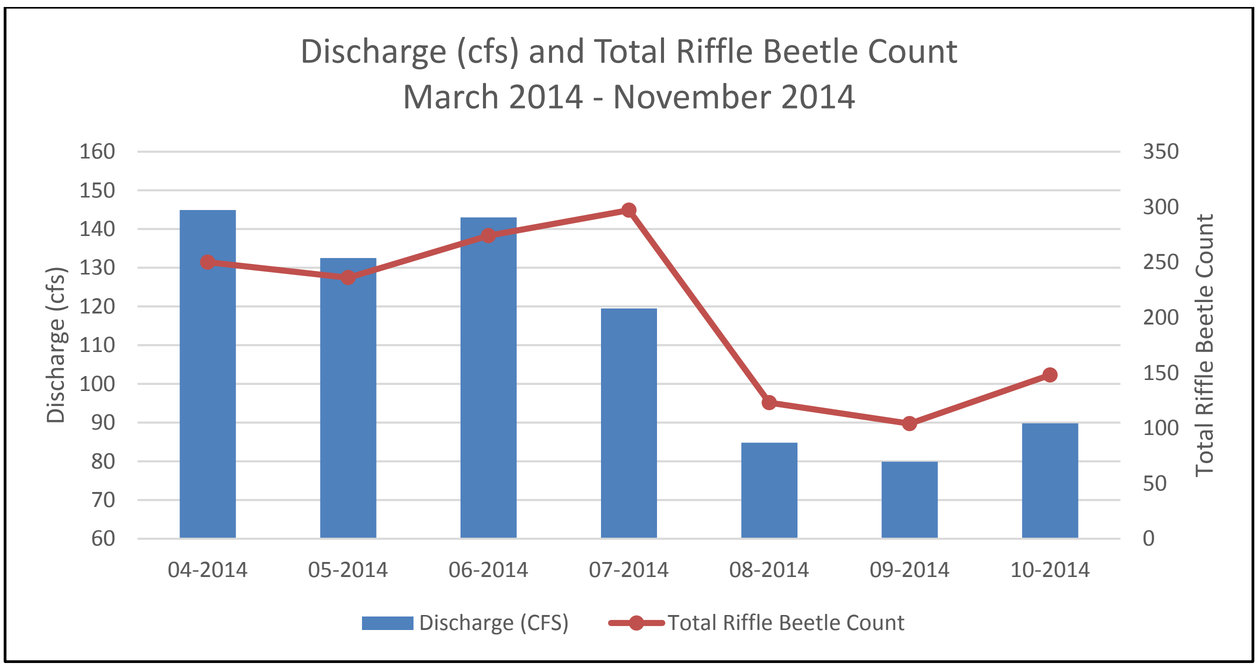

Figure 8. Comal Springs discharge and biomonitoring data for 2014. Biomonitoring data represent totals from Spring Run 3 and Western Shoreline only, due to missing Spring Island data from July and August 2014. Source: USGS 2015; BIO-WEST 2014.

We would expect higher counts from BIO-WEST's biomonitoring data compared to our surveys because their survey sites are entirely in areas the species is known to occur, versus scattered throughout the system at randomly chosen sites. Additionally, their survey sites have been sampled repeatedly using attractive lures, typically with no rest period between bait events, potentially resulting in "trap-happy" beetles (Menkens and Anderson 1988). While the beetle may follow decreasing flow into the subsurface during times of drought, there is likely a threshold reached (determined by how long and/or extensive the drought is) before the population begins to decline.

Possible Sources of Error

Population estimates derived from surveying species within a subset of their habitat can be problematic, and the population estimates we provide herein should be interpreted cautiously. For example, density estimates derived from avian point-counts typically result in an overestimation of density for rare species, while underestimating density for more common species (Howell et al. 2004, Toms et al. 2006, Cimprich 2009, Hunt et al. 2012, Warren et al. 2013). These imprecise estimates can lead to erroneous conclusions about a species' status and response to threats, which can be detrimental to conservation planning and recovery.

There are numerous possible sources of error in the population estimate derived from our study. We ran our model on an interpolated dataset that was collected in less than one month over the course of only three survey events during one of the worst droughts on record. The population estimate we generated for our 95 survey sites was 247; however, at best this only represents approximately one out of every three spring sites in the system at that time. It is possible that we missed potential sites to survey (reducing our sample frame), especially in areas of diffuse upwellings where the flow may have been so low that it was not detectable. If this occurred, it would mean that we sampled fewer than one out of three of the springs, leading to a lower system-wide population estimate. When we extrapolated our estimate to obtain a system-wide abundance of 741 beetles, we also assumed that the remaining sites in the system were identical to our sites; however, this is also unlikely to be true. It is important to reiterate that our methods included skipping one randomly chosen site that overlapped with BIO-WEST's low-flow monitoring sites, likely leading to bias due to all sites not being equally available for sampling. However, this only occurred once and any introduced bias would be minimal.

Additionally, because we did not use mark-recapture techniques, we do not know if the beetles we captured after the first sampling period were the same individuals, newly captured individuals, or a mixture of recaptures and new captures. Each of these scenarios would produce a different population estimate; thus, we will never have a reasonably accurate estimate of the size of their population at Comal Springs or the population's response to low flow events without performing a mark-recapture study under various flow regimes. The existing population estimate methods are based on an entirely different methodology (Recon et al. 2012); therefore, we expect the two methods to provide different results. Indeed our estimates herein are much lower. Unfortunately, those existing methods also employ many assumptions and extrapolations, and therefore have a similar set of problems. The size and morphology of the beetle does not lend itself to successfully marking individuals. Future researchers could use a time series of abundance estimates compared with flow to get a better idea of where the true variability lies.

The models used for our population estimate were based on low-flow conditions, and the population estimate provided should not be extrapolated to estimate the population size during times of higher flow. There were fewer springs in the system at the time we surveyed compared to times of average or above average flow, and it is unlikely that springs at the time we surveyed would have the same characteristics (or even be in the same exact location) as springs flowing during average flow regimes. At best, the population estimate provided should be considered an approximation for the specific time period we sampled that is likely inaccurate due to the study's limited sampling design and compressed time frame during one of the lowest flow regimes on record. We suggest repeating this study under various flow conditions to investigate variability due to reduced sampling areas and/or potential subsurface habitat use during times of low flow.

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