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ORIGINAL ARTICLE

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Population assessment and long-term analysis of the endangered Comal Springs riffle beetle, *Heterelmis comalensis*

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Abstract

1. The Comal Springs riffle beetle (*Heterelmis comalensis*; CSRB; Coleoptera: Elmidae) is a flightless species endemic to two springs flowing from the Edwards Aquifer in central Texas. It was listed as federally endangered primarily due to its limited distribution and the threat of water over-extraction from the aquifer.
2. The listing led to the initiation of a research programme that has improved our understanding of the species' biology and the establishment of captive populations. The primary CSRB population in Comal Springs and a co-occurring cosmopolitan species, *Microcylloepus pusillus* (Coleoptera: Elmidae), have been monitored semiannually since 2004.
3. Our overall goal was to investigate responses to environmental variation using an intensive 1-year population study and long-term data.
4. The population assessment was more spatially and temporally intensive than biomonitoring and was initiated to assess the distribution of the CSRB, determine which environmental covariates relate to relative abundance and develop relative abundance estimates to inform biomonitoring.
5. The population study indicated that spring flow was the primary driver of adult and larval CSRB populations, suggesting that lower spring flows lead to lower populations.
6. CSRB abundance was also positively associated with biofilm coverage, although its effect size was smaller than that of spring flow. *Microcylloepus pusillus* was not affected by spring flow or biofilm, emphasizing the unique ecological niche of the CSRB and the importance of maintaining spring flow through droughts to support populations.
7. Model results were adapted into a framework for an annual assessment of populations using biomonitoring data. Long-term monitoring data suggest that the abundances of both species have declined over the past two decades, but *M. pusillus* has declined beyond the variation explained by included environmental factors.

KEYWORDS
aquatic insects, Coleoptera, Elmidae, endangered species, insect conservation, invertebrate, long-term monitoring, narrow endemism, relative abundance

INTRODUCTION

Conservation of invertebrates has long lagged behind the attention given to other taxa. Invertebrate conservation faces several impediments such as a lack of knowledge regarding species' distributions, abundance and ecology, as well as a lack of will among the public and government to protect them (Cardoso et al., 2011; Strayer, 2006). This has been particularly true among most freshwater insects, which while commonly used in water quality monitoring programmes (Bonada et al., 2006), still suffer from a lack of awareness, knowledge and appropriate taxon-specific monitoring that can be used to inform conservation (Collier et al., 2016). One of the reasons for a lack of focus on aquatic insects is that they may be considered protected under the umbrella of other focal species in an ecosystem (Kalinkat et al., 2017; Muñoz, 2007), although there are some systems where aquatic insects themselves may serve as umbrella species (Whiteman & Sites, 2008). In the continental United States, only seven aquatic insect species have been federally listed: three beetles (Coleoptera), two stoneflies (Plecoptera), one dragonfly (Odonata) and one true bug (Hemiptera). All of these species have very restricted distributions or occupy narrow habitats at high risk of human impacts (e.g., Green et al., 2022; Monroe & Britten, 2014; Polhemus, 1993).

An exception to the lack of conservation focus on aquatic insects occurs in the Edwards Aquifer, which underlies central Texas and provides water for millions of people (Sharp et al., 2019). It is a karst aquifer with dozens of endemic species across several freshwater taxonomic groups, both in surface springs and in subterranean habitats (Bowles & Arsuffi, 1993; Krejca & Reddell, 2019). Two endemic beetle species are listed as federally endangered, the Comal Springs riffle beetle (*Heterelmis comalensis*; CSRB; Coleoptera: Elmidae) and the Comal Springs dryopid beetle (*Stygoparnus comalensis*; Dryopidae). The CSRB was initially described as a species endemic to the Comal Springs system in Comal County (Bosse et al., 1988) and was later found 28 km away in San Marcos Springs, Hays County (Gibson et al., 2008). Only the Comal Springs population is considered here because it is much larger, better understood, and covered under the Edwards Aquifer Habitat Conservation Plan (EAHCP).

CSRB are brachypterous, meaning they have incompletely developed, non-functional wings (Bosse et al., 1988). The loss of wings is likely an adaptation to conserving resources (Zera & Denno, 1997) in the stable environmental conditions provided by what historically has been the largest spring complex by discharge in Texas (Brune, 2002). In turn, CSRB are reliant on this stable spring system and do not occur in other smaller springs throughout central Texas that sometimes support their own endemic aquatic species (Barr et al., 2023; Gibson et al., 2008; Hutchins, 2018). In 1997, this led to the listing of CSRB as federally endangered, with the species threatened by the combination of their limited distribution and increasing human development above the Edwards Aquifer (USFWS, 1997). In particular, the most prominent threat is excessive water withdrawal from the aquifer (USFWS, 1997). There is also a recognized risk to the species if groundwater contamination were to occur (Bowles & Arsuffi, 1993; USFWS, 1997), although evidence for contamination of the aquifer has been minimal (Hutchins, 2018). During the drought of record in the 1950s when the Comal Springs system ceased surface flow, CSRB presumably survived in hyporheic habitats, but it is unknown how adversely the species was affected. A total of 22 ha of surface habitat that has been designated as CSRB critical habitat—habitat that is essential for a species' conservation—in the Comal Springs system (USFWS, 2013). A recent review of the species found no need for a status change, stating that it remains at risk due to water withdrawals and land use changes (USFWS, 2024).

Following the listing of CSRB, a biological monitoring programme with standardized semiannual sampling was initiated in 2004. Further conservation actions have been taken as part of the EAHCP such as restrictions on water withdrawals, restoration of surrounding terrestrial habitats and limits on human recreation in spring habitats (Payne et al., 2019). An initial field survey (Bowles et al., 2003) found no clear microhabitat associations of the species, but an association with habitats near spring outlets was later confirmed (Cooke et al., 2015). A wide range of other studies has been undertaken to understand the species, which along with several unpublished reports and theses, includes publications on its diet (Nair et al., 2021), microbiome (Mays et al., 2021), responses to environmental conditions in captivity (Nair et al., 2023) and its life history (Fries, 2003; Huston & Gibson, 2015; Kosnicki, 2022). The full extent of work on the species was summarized by the U.S. Fish and Wildlife Service (2024). Life history research has led to the establishment of reproducing captive populations that could be used for reintroduction if a catastrophic event were to occur in the wild (USFWS, 2022).

Despite 21 years of CSRB biomonitoring and extensive study of other aspects of its biology, little remains known about the environmental factors that affect CSRB habitat occupancy and abundance in the wild. Mark-recapture has proven ineffective (Huston et al., 2015), females are seemingly continuously iteroparous (Kosnicki, 2022) and there is a lack of seasonal phenological differences in the presence and abundance of each life stage (Bowles et al., 2003). These factors, along with the potential effects of spring habitat desiccation on CSRB, have made it difficult to study and analyse this species using many of the traditional methods used for assessing species occupancy and population size (e.g., Royle, 2004).

To assess the current status of CSRB and their relationship to environmental factors, we conducted a 1-year intensive CSRB population study. This study was designed to assess CSRB occurrence and abundance across the Comal Springs system using more rigorous and spatially expansive surveys than were used previously or in semiannual biomonitoring, while repeating surveys at the same sites multiple times over the course of 1 year, and quantifying potential environmental covariates. The primary objectives of the population study were to (1) determine how environmental covariates relate to CSRB abundance; (2) develop model-based relative abundance and apparent occupancy estimates that can be used to inform the status of the species as it relates to environmental factors during semiannual biological monitoring; and (3) compare covariate effects on CSRB to those on *M. pusillus*. We subsequently examined all long-term CSRB and *M. pusillus* data collected with standardized methods to determine

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(1) what are the long-term trends of the populations of these two beetle species in Comal Springs and (2) what are the relationships between system-wide environmental covariates and beetle populations over the past 21 years?

METHODS

Study system

The Comal Springs system is a complex of springs divided into several different spring areas (Figure 1; referred to here as 'localities'). Spring Run 1 is at the highest elevation and the first to dry when aquifer levels drop; Spring Run 2 is the smallest area with a few springs in its upper half that also partially dries when flows drop; Spring Run 3 has more spring inputs and more reliably maintains flow under drought conditions than Spring Runs 1 and 2. The Western Shoreline is a system of mostly terrestrial margin springs along the western shoreline of Landa Lake (an impoundment of Comal Springs); it also includes several deeper upwelling sites at the bottom of the lake. The Spring Island locality consists of a complex of upwellings and terrestrial margin springs surrounding and to the north of an island (Spring Island) in the lake; this area has been the most reliable at maintaining wetted spring sites and noticeable spring flow during recent droughts. An additional locality, the Upper Spring Run, sits at the northern end of the Comal Springs system, but springs there ceased noticeable flow during the drought. While we sampled the Upper Spring Run for the population assessment, it is otherwise not included here because we did not find any CSRB there and only a few individuals have ever been reported from that locality (Bowles et al., 2003; J. R. Gibson, personal communication). Spring Run 3, the Western Shoreline and Spring Island localities have historically been the focus of CSRB biomonitoring efforts.

The population assessment coincided with one of the periods of lowest total-system spring flow in 30 years. Although total-system spring flow during this study was generally low, it exhibited considerable variation across all of the study periods, with a maximum of 205 cubic feet per second (cfs) in January 2024 and a minimum of 66 cfs in July 2023 (Appendix A: Figure S1); the long-term average is ~280 cfs. Low spring-flow conditions experienced during the study provided an important opportunity to gain insights into the potential impacts of climate change, continued land use change and potential long-term declines in spring flow.

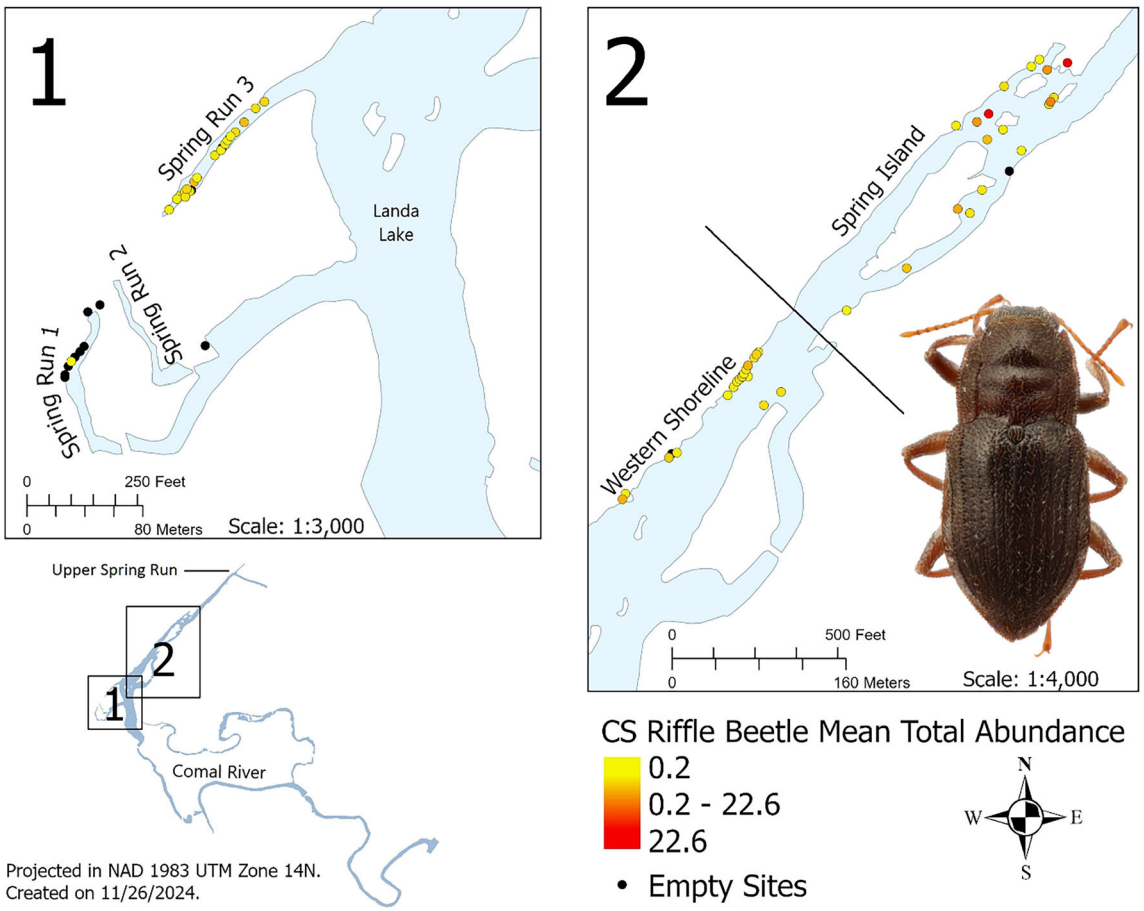

FIGURE 1 Sample sites and locations where any individuals either Comal Springs riffle beetle (CSRB) life stage were observed (empty sites = no CSRB found) and the mean total (adults + larvae) of CSRB per lure across the duration of the population assessment. In (2), the black line separates the Western Shoreline sites from the Spring Island sites and the inset image is an adult *Heterelmis comalensis*.

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Sampling methods

Long-term monitoring for CSRB began in 2004 using pieces of white cotton cloth that were placed under rocks in spring openings. This was modified and standardized into a formal cotton lure methodology in 2014 following EAHCP implementation: lures consist of a 15 cm × 15 cm square piece of 200 thread count cloth (60% cotton and 40% polyester) folded by thirds and placed into a 4 cm × 4 cm cage made from galvanized wire (Edwards Aquifer Authority, 2016). At spring openings that had noticeable spring flow, lures were placed just below the substrate-water interface (predominantly the hyporheic zone) and covered with rocks to hold in place and block light from the lure (CSRB avoid light; Cooke et al., 2015).

Lures were left in situ for approximately 30 days, allowing for the colonization and growth of biofilm on each lure (Appendix A: Figure S6), which serves as a food source and potential attractant that encourages colonization of the lures by CSRB. Longer deployment of lures often leads to degraded lures that experience greater/earlier biofilm colonization and in turn fewer CSRB since the lure no longer retains its structure (Huston et al., 2015). Upon retrieval, any lures that were disturbed (e.g., dug up or cloth removed from cage), exposed to light, experienced heavy sedimentation (typically from heavy precipitation), dried due to declining water levels or were otherwise lost, were excluded from analyses.

Upon lure retrieval, adult and larval CSRB and a similarly sized, co-occurring non-listed species, *Microcylloepus pusillus* (Coleoptera: Elmidae) were identified and counted. *Microcylloepus pusillus* is a species that ranges widely across North America (Jäch et al., 2016) and, in addition to spring habitats, it also occupies non-spring-associated rocky streams throughout central Texas (Burke, 1963). *Microcylloepus pusillus* can possess fully developed wings, although individuals and populations with incompletely developed wings are common (Shepard, 2019) and the role these wing morphs may play in spring systems has not been studied. Most of the invertebrates were returned to their spring of origin after enumeration; CSRB were occasionally removed for other projects or for incorporation into the EAHCP Refugia Program. Given low rates of recapture (<1%; Huston et al., 2015), it is not expected that this limited removal of CSRB from the system had any noticeable impact on subsequent counts.

Population assessment

Sampling design and data collection

Sampling during the CSRB population assessment was conducted using cotton lures at 83 sites across the Comal Springs system during five 1-month sampling periods from April 2023 until May 2024. Sites were distributed across four localities (Figure 1): Spring Run 1 (10 sites), Spring Run 3 (10 sites), Western Shoreline (21 sites) and Spring Island (20 sites). These sites were selected from the three subpopulations proposed by Lucas et al. (2016) based on molecular data: Spring Run 1, Spring Run 3 and Western Shoreline + Spring Island. We planned the sampling design around these subpopulations by balancing the availability of active springs and the spatial coverage of sites at each locality. Spring Run 2 (Figure 1) was excluded because it was expected to have fewer active springs and was considered to be recovering from restoration activities. For reasons mentioned above, sampling efforts from the Upper Spring Run were not included in this population assessment. Further details on the spatial and temporal distribution of sampling are provided in Appendix A.

Several spring-level, lure-level and higher-level (locality or total-system) covariates were measured or calculated at the time lures were set and/or retrieved. These included water temperature, dissolved oxygen, conductivity, pH, water depth, lure depth, average substrate size, presence of organic material, biofilm coverage on lures, number of days lures were set and spring flow at the site, locality and total-system levels. Details on how all covariates were measured or calculated are presented in Appendices A and B. For all of the covariates, we visually explored and interpreted the data, incorporated knowledge of the ecology of the Comal Springs system and preliminarily explored models including most covariates with the 'mgcv' package in R (R Core Team, 2025; Wood, 2011) (see Appendix A). Final covariates were then included in the final model formulation and refined for modelling fitting and inference, which only included 30-day local spring flow average and total biofilm, as no other environmental covariates were related to CSRB abundance and many showed no meaningful or consistent variation across the spring system (Appendix A). Final analyses with generalized linear-mixed effects models were conducted on CSRB and *M. pusillus* adults and larvae, developed under a Bayesian framework to better understand what environmental factors influenced patterns of beetle relative abundance and apparent occupancy using the 'jagsUI' R package (Kellner, 2024). In short, each model was fit with a Poisson error distribution and included two random effects terms: (1) site-level intercepts to account for additional sources of variation across sites that were not explained by covariates; and (2) extra-dispersion term describing residual variation at the sample level not explained by the random intercepts or covariate effects. Coefficients for each covariate were modelled as fixed effects. Full descriptions on model formulation and methods used for model evaluation and inference are presented in Appendix B.

Long-term data

We gathered all data with verifiable counts, dates and locations that were collected using cotton lures or the preceding cloth method from 2004 through November 2025. There was a total of 2290 samples, and over half of the data (1222 samples) were collected during semi-annual biomonitoring. Additional monitoring was conducted when total system spring flow declined below 120 cfs; this accounted for 422 samples. The two other main data sources were the population assessment (215 samples not including samples shared with biomonitoring) and collections by the USFWS San Marcos Aquatic Resources Center (253 samples). The remaining data included 89 samples from Cooke et al. (2015) and Huston et al. (2015), and 89 samples

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from unpublished studies. Most samples were distributed among the three biomonitoring localities: Spring Run 3 (785), Spring Island (676), Western Shoreline (667). Fewer samples were collected from Spring Run 1 (90) and Spring Run 2 (72).

We reviewed the location at which each sample was collected and, when possible, connected each sample to the exact spring opening. However, in many cases the exact sites were not clear but each spring could be located within certain sectors of each locality (e.g., Appendix A: Figures S8–S10). The abundance of CSRB adults and *M. pusillus* adults was analysed using generalized additive mixed effects models with the 'mgcv' package (Wood, 2011) with a negative binomial distribution. Because environmental covariates at each spring site were not consistently recorded across much of the time series, we only included total system covariates and 30-day local spring flow average (see Appendix B) in models. Total system covariates were 30-day precipitation, 180-day average total-system discharge and 180-day average air temperature. Covariates over 30 days represent effects that occurred during the study period and 180-day covariates represent potential effects over the development time of a beetle from egg to the adult stage. Although development times of *M. pusillus* are not known, the CSRB larval period lasts as for little as 12 weeks, while larval periods of 6 months or less are commonly observed among other elmids (Elliott, 2008; United States Fish and Wildlife Service, 2024; White, 1978). The 30-day local spring flow covariate was included as a smooth term by locality to account for potential locality-specific non-linear relationships with spring flow.

An initial model was run with year but without environmental covariates to test for an overall temporal trend; a full model including all covariates except year was developed to examine potential effects of environmental variables; a final model was run using the covariate model as an offset with only year as a continuous variable to determine if long-term trends persisted after accounting for known covariates. All continuous covariates were standardized and, if necessary, natural-log or square-root transformed. Site, sampling method (cotton lure or cotton cloth), and sample project were included as random effects. The number of days lures were deployed in the system was included as an offset. The ordinal day of the year was included as a smooth term to account for variation in time of sample collection across the years and potential seasonality.

RESULTS

Population assessment

Beetles recorded

Out of a total of 404 lures set during the study, 14 were lost due to disturbance and 38 were dry at the time of retrieval, resulting in 352 lures (samples) with complete data. All dry lures were found during the summer of 2023 when total-system spring flow dropped dramatically from the start of the sampling period (116 cfs) to the end (66 cfs; Appendix A: Figure S1). In total, over 600 adults of both CSRB and *M. pusillus* were found, with the highest numbers around Spring Island, fewer at Spring Run 3 and the Western Shoreline and only a single CSRB in Spring Run 1 (Appendix A: Table S3; Figure 1). Trends in the mean abundance of both life stages of CSRB and *M. pusillus* did not show any clear temporal variation in abundance that could be indicative of phenological differences in abundance across seasons (Appendix A: Figures S11–S14).

Habitat covariates

Water quality variables showed little variation across the study and no clear relationships with beetle abundance, as was expected in a stable spring system (Appendix A: Figures S16–S18). Lures were set at depths from just below the water surface (~2 cm) to over 2 m deep (Appendix A: Figure S19), and CSRB and *M. pusillus* were found at sites across those ranges of depths. Substrate varied from sites that were nearly all boulder/bedrock to those that were all silt/sand (Appendix A: Figure S20); beetles were found at sites across the range of substrate sizes. While preliminary comparisons suggested that higher numbers of beetles may be found on smaller substrate sizes, this was tied to higher CSRB abundances and smaller substrate sizes at Spring Island sites, which were the most stable (consistently under water and stable spring flow) throughout the study.

Site-level spring flow varied by site and locality across the five sampling periods (Appendix A: Figure S21), but there was no clear relationship between site-level spring flow and beetle abundance. Lures were always set in flowing springs, and beetles were found across the range of site-level flows observed. Initial analyses suggested a possible relationship between total-system spring flow and beetle abundance, but this was refined into a calculation of local spring flow wherein the components of total-system spring flow were attributed to localities within the system (Appendix B). Local spring flow also accounted for potential effects of spatial group factors observed in initial data exploration.

There was no clear effect of the presence of any organic matter-type in the spring area on the abundance of beetles (Appendix A: Figure S22). Estimated biofilm coverage on the lures varied widely among samples with no clear temporal or spatial patterns (Appendix A: Figure S23). However, initial comparisons suggested a potential positive relationship between biofilm coverage and CSRB abundance. Biofilm coverage and local spring flow were the two covariates included in the final analyses.

Statistical results

Average relative abundances represent site-level intercepts on the original response scale (counts/lure), which were used to derive apparent occupancy probability (Figure 2). Differences in intercept estimates on the relative abundance scale are described here and apparent occupancy between sites is included in Figure 2 for comparison. Intercept estimates were zero at Spring Run 1 for each species-life stage combination and were also zero for *M. pusillus* larvae at Spring Run 3. Site-level intercept estimates (90% Bayesian Credible

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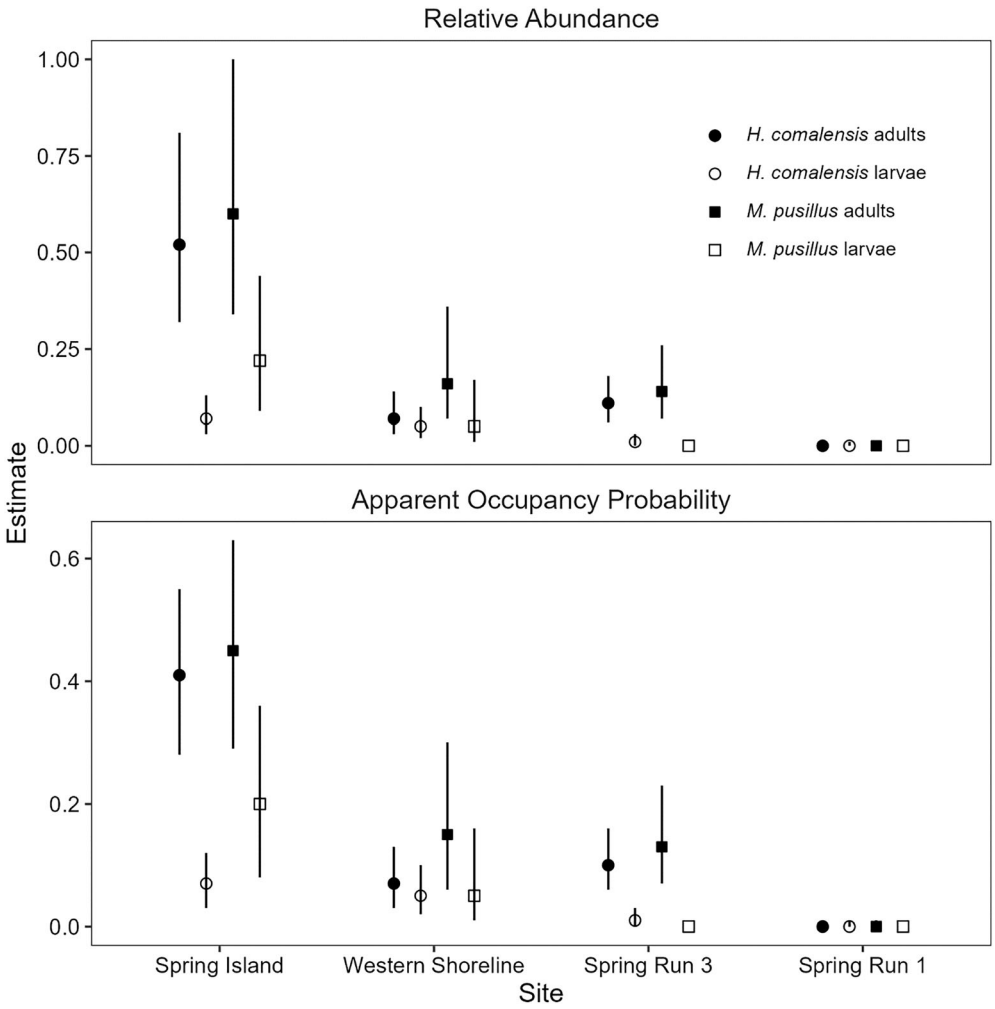

FIGURE 2 Generalized linear mixed effects model estimates of average relative abundance and apparent occupancy probability at each site. Average relative abundances represent site-level intercepts on the original response scale (counts/lure), which were used to derive apparent occupancy probability. Error bars denote 90% Bayesian credible intervals.

Interval [BCI]) for CSRB adults were 0.52 (0.32–0.81) at Spring Island, 0.07 (0.03–0.14) at the Western Shoreline and 0.11 (0.06–0.18) at Spring Run 3. Credible intervals for Spring Island did not overlap with other sites, suggesting it is highly probable that average relative abundance was greater at this site relative to the others. Intercepts for CSRB larvae, *M. pusillus* adults and *M. pusillus* larvae also illustrated greater average relative abundance at Spring Island. However, broad and overlapping credible intervals indicated uncertainty in whether average relative abundance truly differed between sites (Figure 2).

For each GLMM, random-effects standard deviations were higher for site compared to the extra-residual term. This indicated there were larger differences in relative abundance between sites that weren't accounted for by the model covariates relative to the observation-level (i.e., lure). Estimates (90% BCI) of 30-day local spring flow average effect size was 0.14 (0.06–0.21) for CSRB adults and 0.30 (0.22–0.40) for CSRB larvae. Slope estimates for the effect size of total biofilm on CSRB relative abundance were 0.04 (0.03–0.05) for adults and 0.03 (0.02–0.05) for juveniles. Bayesian credible intervals for CSRB adult and larval slopes did not overlap with zero, meaning there was a 0.9 probability that both covariates had a positive effect on relative abundance. For *M. pusillus*, local spring flow and total biofilm effect sizes were 0.03 (−0.06–0.12) and 0.01 (0.00–0.03) for adults and −0.13 (−0.29–0.02) and 0.00 (−0.02–0.02) for juveniles, respectively. In contrast to CSRB, the breadth of BCIs for both *M. pusillus* life stages failed to reject zero as a plausible effect size for each covariate (Table 1).

Covariate relationships of CSRB relative abundance are presented for adults in Figure 3. Relative abundance and apparent occupancy probability relationships are presented for adults and larvae in Appendix B: Figure S27. The extent of 30-day local spring flow averages on the x-axis of each panel encompasses the range of discharge magnitudes observed at each site over the study period. Since the effect size of local spring flow was fixed across sites, maximum predictions of relative abundance and apparent occupancy probability occurred at Spring Island and the Western Shoreline because they exhibited the greatest maximum 30-day local spring flow averages. Consequently, as local spring flow decreased from its maximum to minimum magnitude, state variables for both life stages were

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TABLE 1 Estimates (Est) and Bayesian credible intervals (BCI) of parameters used to fit generalized linear mixed effects model of relative abundance in the population assessment.

	CSRB adults		CSRB larvae		MIPU adults		MIPU larvae	
Parameter	**Est**	**BCI**	**Est**	**BCI**	**Est**	**BCI**	**Est**	**BCI**
Intercepts								
Spring Island	−0.65	(−1.13 to −0.22)	−2.68	(−3.44 to −2.04)	−0.50	(−1.08 to 0.00)	−1.52	(−2.44 to −0.81)
Western Shoreline	−2.68	(−3.43 to −2.00)	−3.03	(−3.89 to −2.28)	−1.81	(−2.72 to −1.02)	−2.94	(−4.49 to −1.75)
Spring Run 3	−2.22	(−2.76 to −1.72)	−4.38	(−5.39 to −3.53)	−1.98	(−2.68 to −1.37)	−11.26	(−22.92 to −6.57)
Spring Run 1	−7.62	(−16.57 to −4.59)	−7.06	(−15.44 to −4.38)	−8.01	(−17.02 to −4.79)	−10.70	(−22.55 to −5.81)
Population-level intercept	−3.29	(−8.49–0.62)	−4.25	(−8.75 to −1.29)	−3.07	(−8.54–1.04)	−6.78	(−14.37 to −1.35)
Covariate effect size								
30-day local spring flow average	0.14	(0.06–0.21)	0.30	(0.22–0.40)	0.03	(−0.06–0.12)	−0.13	(−0.29–0.02)
Percent total biofilm	0.04	(0.03–0.05)	0.03	(0.02–0.05)	0.01	(0.00–0.03)	0.00	(−0.02–0.02)
Random effects standard deviation								
Site	4.32	(1.65–9.09)	3.01	(0.94–8.47)	4.67	(1.84–9.17)	6.56	(2.93–9.64)
Extra-residual	1.55	(1.33–1.82)	1.56	(1.28–1.9)	2.02	(1.75–2.35)	2.25	(1.75–2.98)

Abbreviations: CSRB, Comal Springs riffle beetle, MIPU = Microcylloepus pusillus.

expected to decrease by about 90%–100% at Spring Island and the Western Shoreline. In contrast, at Spring Run 3, CSRB adult state variables were expected to decrease by about 85% compared to an expected decline near 100% for CSRB larvae. It is also important to note that relative abundance predictions for CSRB larvae at Western Shoreline showed an exponential increase from about 1 to 139 counts/lure as local spring flow increased from 85 to 100 cfs. Maximum predictions of CSRB larvae at this site were much higher than the observed maximum (14 counts/lure), suggesting the slope estimate of local spring flow was not realistic for this life stage (Figure 3; Appendix B: Figure S27).

Given that total biofilm was quantified as a percentage, its functional relationship with CSRB adults and larvae was more consistent between sites relative to local spring flow. Differences in maximum predictions were therefore governed by estimates of each site's intercept. As total biofilm increased from 0% to 100%, relative abundance and apparent occupancy probability were expected to increase about 97% for both life stages. Lastly, predicted values of relative abundance and apparent occupancy were very similar for all fitted functions except for CSRB adults at Spring Island, which illustrated that as relative abundance approached 3.0 counts/lure, apparent occupancy probability approached 1.0 (Figure 3; Appendix B: Figure S28).

Long-term analysis

Our long-term dataset from 2004 to 2025 included 18,448 adult CSRB and 14,283 adult *M. pusillus*. Most CSRB were collected from Spring Run 3 ($N = 7675$), which historically supported the highest mean number per sample but has been consistently low during the drought of 2022–2025 (Figure 4e). The Western Shoreline ($N = 4969$) had the second-most total number of CSRB, but numbers declined during the droughts of 2014 and 2015 before rebounding during 2016 but remaining low since (Figure 4c). Mean abundances have remained relatively stable over time at Spring Island (Figure 4a: $N = 4229$), but the relatively few samples and beetles collected from Spring Run 2 ($N = 940$) and Spring Run 1 ($N = 635$) also indicate very low numbers during the drought of 2022 of 2025 (Figure 4g). The highest abundances of *M. pusillus* were found at Spring Island (10,693), where mean abundances have declined substantially over the past 20 years (Figure 4b). Fewer *M. pusillus* were found at Spring Run 3 (Figure 4f; $N = 2733$), Western Shoreline (Figure 4d; $N = 704$), Spring Run 1 (Figure 4h; $N = 118$) and Spring Run 2 ($N = 35$).

Initial analyses that excluded environmental covariates indicated significant temporal declines in the abundances of both CSRB and *M. pusillus* over the past 21 years (Table 2). In full analyses, both species had positive relationships with 180-day total system discharge and precipitation, but negative relationships with 180-day average air temperature. All smooth terms and random effects were significant, with significant relationships between 30-day local spring flow average in each locality except Spring Run 2 for CSRB and Spring Run 1 for *M. pusillus* (Figures S29 and S30). After accounting for covariate effects, CSRB showed no long-term linear trend in abundance, but year was still significant for *M. pusillus*, although the magnitude of the coefficient was greatly reduced, still indicating that some of their declines in abundance remain unexplained.

DISCUSSION

For the Comal Springs riffle beetle and in the Comal Springs system, the key results of this study are (1) both adult and larval CSRB are

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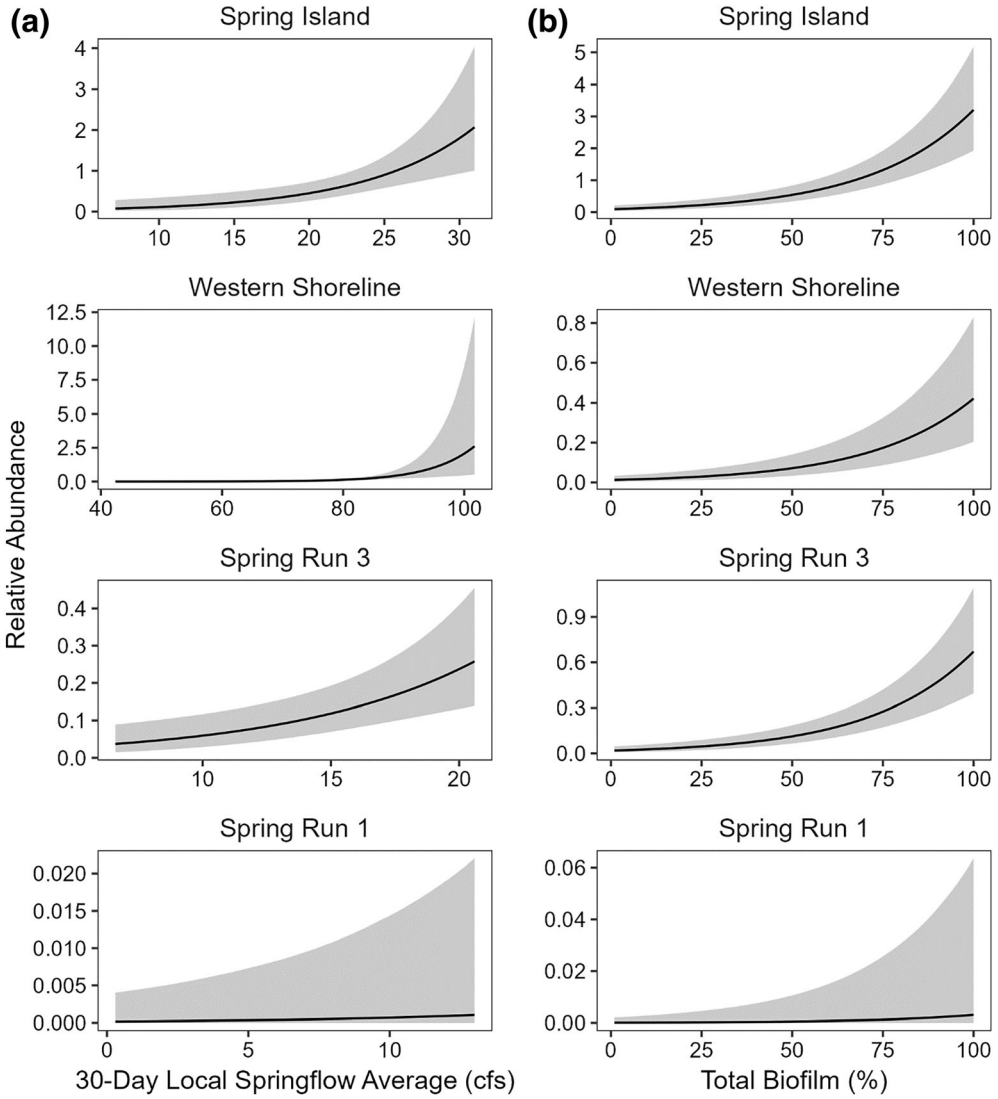

FIGURE 3 Relative abundance relationships with 30-day local spring flow average (a) and total biofilm average (b) among sites for adult *Heterelmis comalensis*. Solid lines represent the line of best fit and grey polygons denote 90% Bayesian credible intervals.

readily found throughout the year with no apparent seasonality and across nearly all active spring sites in Spring Run 3, Spring Island and the Western Shoreline, while the lack of spring flow in Spring Run 1 indicates that locality may not currently support a population and less is known about the species in Spring Run 2; (2) local spring flow had a large positive effect on CSRB relative abundance and apparent occupancy across each of these three localities, while biofilm also had a positive effect, which contrasted with the lack of response to spring flow or biofilm by the non-spring species *M. pusillus*; and (3) the current collection techniques and models developed here can be used to track and assess CSRB populations over time in the biomonitoring programme under the framework provided in Appendix C. These results illustrate the importance of having an effective monitoring programme so that changes to populations can be detected, environmental effects can be understood and further conservation actions can be planned based on data.

Spring flow was the most important environmental variable in determining the relative abundance and apparent occupancy of CSRB. Elmidae are adapted to clinging to substrates in flowing water conditions (Elliott, 2008), so it is generally expected that species in this family will be found in, and are more common when, there is persistently flowing water. In particular, spring flow is important for CSRB, but water flow in general, not necessarily spring flow, is important for *M. pusillus*. The morphology and ecology of elmids, along with their relative lack of occurrence in drift nets during biological monitoring over the past 20 years (BIO-WEST, 2024), indicates that their occurrence on lures is an active behavioural response wherein they select habitats with flow and food (biofilm). Additionally, initial data suggest that CSRB primarily occur in the hyporheic zone at the water-substrate interface, even if there may be spring flow deeper in the substrate (BIO-WEST, unpublished data), so CSRB are not being passively caught in lures after being

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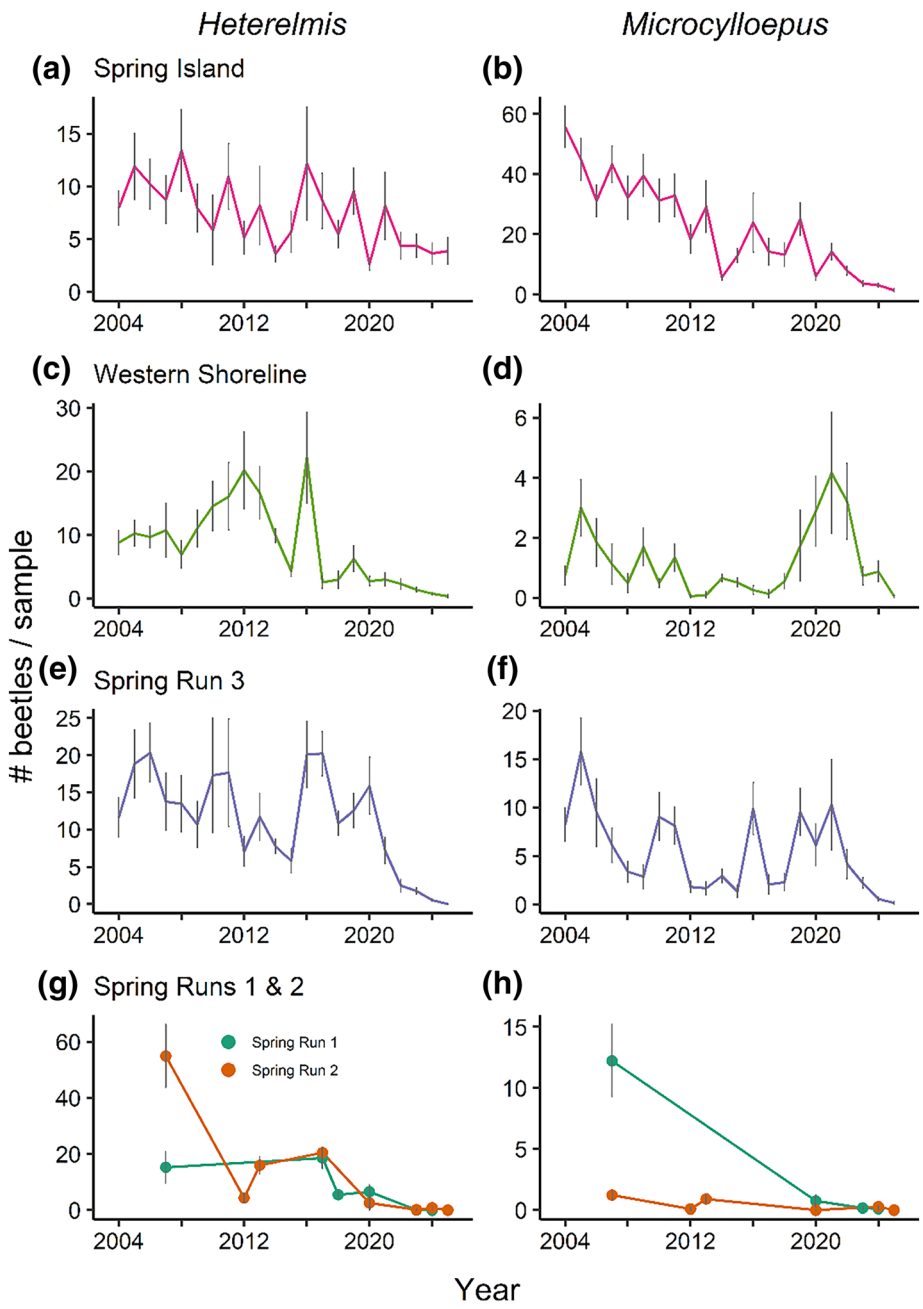

FIGURE 4 Mean (±SE) number of adult *Heterelmis comalensis* (left column) and adult *Microcylloepus pusillus* (right column) per sample during each year from 2004 to 2025 in the three primary sampling localities (Spring Island, Western Shoreline, Spring Run 3), plus limited data on samples collected from Springs Runs 1 and 2.

swept away in flowing water. The positive relationship between CSRB and spring flow, seen in both the population study and the full time series, illustrates the importance of maintaining spring flow for the persistence of this species, especially as human groundwater demands increase within the Edwards Aquifer. Rather than a local movement of CSRB from our monitored sites to deeper locations during drought, recovery following drought seems more likely to occur through reproduction than only migration. Spring Run 1 is likely the exception as it is the only locality where we have been unable to detect CSRB during the ongoing drought, so recovery there may depend on migration and reproduction; a genetic analysis comparing subpopulations could help to elucidate these questions.

While the models suggest we should expect more CSRB within Comal Springs where and when there is locally more spring flow, these models should not and cannot, be expanded to other spring systems in the region. The models apply at the local level (within localities of Comal Springs) and do not apply to predicting whether other springs outside the system could support a translocated CSRB population. As the historically largest spring system by volume in Texas, Comal Springs likely had a unique role in allowing for the evolution of

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TABLE 2 Results of generalized additive mixed effects models on long-term datasets for adult *Heterelmis comalensis* and *Microcylloepus pusillus*.

	H. comalensis				*M. pusillus*			
	Est.	**SE**	***z***	***p***	**Est.**	**SE**	***z***	***p***
Year								
Initial model	−0.892	0.061	−14.6	<0.0001	−0.705	0.043	−16.2	<0.0001
After covariates	−0.130	0.249	−0.5	0.60	−0.537	0.250	−2.1	0.032
Full covariate model								
Parametric terms								
Discharge (180-day)	0.886	0.115	7.8	<0.0001	1.152	0.141	8.2	<0.0001
Temperature (180-day)	−1.060	0.154	−6.9	<0.0001	−0.785	0.190	−4.1	<0.0001
Precipitation (30-day)	0.131	0.039	3.2	0.0009	0.143	0.045	3.2	0.0014
Smooth terms	**edf**	**Ref df**	χ^2	***p***	**edf**	**Ref df**	χ^2	***p***
Site (RE)	113.8	349.0	404.7	<0.0001	100.5	220.0	382.7	<0.0001
Project (RE)	6.8	8.0	179.1	<0.0001	3.5	6.0	27.4	0.0002
Method (RE)	1.0	1.0	110.9	<0.0001	1.0	1.0	44.0	<0.0001
Day of year (TP)	6.2	7.4	61.1	<0.0001	7.9	8.6	64.8	<0.0001
30-D SF:SI (TP)	1.0	1.0	31.7	<0.0001	4.5	5.2	49.0	<0.0001
30-D SF:SR1 (TP)	2.3	2.7	8.9	0.012	1.0	1.0	0.9	0.33
30-D SF: SR2 (TP)	1.5	1.7	4.5	0.14	2.0	2.2	8.1	0.023
30-D SF:SR3 (TP)	4.2	4.8	31.9	<0.0001	3.8	4.5	37.6	<0.0001
30-D SF:WS (TP)	4.7	5.5	41.9	<0.0001	3.4	4.1	48.8	<0.0001

Note: The top two shaded year rows are from the initial model without any other environmental covariates and the model that included all other terms as an offset (examining for trends after accounting for the covariates); all other rows are from the final model. 30-D SF indicates 30-day average local spring flow for each of the five localities (SI, Spring Island; SR1, Spring Run 1; SR2, Spring Run 2; SR3, Spring Run 3; WS, Western Shoreline).
Abbreviations: RE, random effects; TP, thin plate splines.

this endemic species, while other spring systems have, in many cases, developed their own communities of endemic species (e.g., Barr et al., 2023; Gibson et al., 2008, 2021; Hutchins, 2018). Given the narrow environmental tolerances of CSRB (Cooke et al., 2015), the only other sites similar enough to Comal Springs to likely to support CSRB would be springs in central Texas that are connected to varying degrees to the same aquifer as Comal Springs and more or less similarly affected by changes to aquifer levels (Brune, 2002). The closest major spring to Comal Springs is San Marcos Springs, which has historically been the second-largest spring system in Texas and effectively already supports a small, poorly studied CSRB population. Therefore, translocating CSRB to establish 'backup' populations at other locations is both ill-advised and potentially futile (Novak et al., 2021; Schwartz et al., 2025). Studies of insect translocations have been focused on species that possess some capacity for dispersal (which CSRB does not have) and not among isolated habitat patches (i.e., separate springs) that have their own, potentially competing insect assemblages (Keyghobadi & Daniels, 2025). Additionally, given that there is potentially a unique genetic structure across the system (Lucas et al., 2016), transplanting CSRB across localities within Comal Springs should be avoided (Coates et al., 2018; Crandall et al., 2000; McKay et al., 2005). Conservation efforts should be focus on protecting spring flows and water quality in the aquifer to allow for the long-term persistence of CSRB (and other endemic species) in Comal Springs. The USFWS maintains captive populations of CSRB at two locations, separated by Comal subpopulation, that are intended to be used for reintroduction if a catastrophic event should occur to wild populations.

Other than local spring flow, the lack of clear environmental factors affecting CSRB populations is perhaps a great reflection on the fact that the CSRB is a spring-endemic species and adapted to stable environmental conditions that otherwise show little variation across their range. Variation not accounted for by spring flow and lure biofilm could be a product of stochastic demographic factors that we might never be able to measure, and as a species with a small, highly geographically restricted population, this demographic stochasticity could be highly pronounced, leading to low levels of explained variance (small population paradigm; Caughley, 1994). These effects may even be present across localities in the Comal Springs system, with smaller, potentially more isolated subpopulations in Spring Runs 1 and 2 being more susceptible to stochastic events (Frederiksen et al., 2008; Gabriel & Bürger, 1992) and difficult to model, but we currently lack substantial data from these localities. These subpopulations should be given additional consideration and incorporated into the biomonitoring programme, especially given effects observed during the drought of 2022–2025, the potential loss of the population in

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Spring Run 1, and the potential genetic variation across subpopulations. Continued study and monitoring of CSRB are warranted to accomplish the overall goals of the EAHCP—to track and protect endemic invertebrates over time (EAHCP, 2012).

The long-term data on CSRB and *M. pusillus* populations indicate that there have been system-wide declines over the past 21 years, with statistical declines persisting for *M. pusillus* after accounting for known covariates. While the changes to CSRB populations effectively represent changes to the entire species since they are endemic to the system that is monitored (setting aside the small population in San Marcos Springs), the decline in *M. pusillus* is harder to directly account for. As a wide-ranging species that occurs outside of the spring system (Burke, 1963; Jäch et al., 2016), *M. pusillus* populations within the springs could also be affected by larger-scale processes affecting the species regionally, if macropterous wing morphs allow for migration between populations (Shepard, 2019). Within Comal Springs, declining spring flows, precipitation and warmer temperatures may all play some role in the long-term population changes of both species; these population declines could reverse if environmental conditions improve after the drought of 2022–2025 drought subsides. However, approximately half of the variation in the abundance of these species remained unexplained over the time series, which is not necessarily unexpected for large ecological datasets and may be due to environmental or demographic stochasticity, which is difficult or impossible to measure. Positive effects of precipitation may be in part linked to subsequent increases in spring flow, but rainfall may also transport nutrients from surrounding terrestrial habitats to boost the productivity of spring sites or initiate changes to microbial communities (Liu et al., 2024; Saccò et al., 2021). This in turn could affect biofilm on lures on which the beetles feed on. Excessive local precipitation could result in large amounts of sediment being washed into the spring system, and this has long been a concern with erosion control efforts having sought to limit erosion through the restoration of native vegetation, the restriction of recreation and the construction of sediment barriers. Other elmid species have been associated with substrates coarser than sand and silt (Fujiwara et al., 2022; Yoshimura, 2012), but there has not been an experimental examination of sedimentation effects on CSRB. However, we did find CSRB at sites dominated by sandy and silty substrates, and we expect that as long as sites maintain spring flow, they should be able to support beetles.

The negative relationships with air temperature of the full time-series for both species are likely at least in part related to warmer 180-day average air temperatures during fall versus spring biomonitoring. Total system spring flows are also typically lower in fall than in spring (U.S. Geological Survey, 2025), meaning there is some inverse covariation between the two. Water temperatures within the springs are stable year-round (although there may be some variation in microhabitats such as along shallower edges), but seasonally warmer air temperatures could correlate with longer day lengths and otherwise higher levels of productivity by aquatic and terrestrial vegetation (tree roots are often in the spring sites) and related changes to microbial communities (Adams et al., 2010). However, long-term increases in air temperature could theoretically directly affect CSRB if temperatures in the springs subsequently increase above the thermal optima of the CSRB (Cooke et al., 2015; Pallarés et al., 2021; Polášková et al., 2022; Ward & Stanford, 1982). Although some pollutants are present in the Edwards Aquifer, to date the aquifer has seemed to be relatively resilient to contamination (Doyle, 2024; Hutchins, 2018), but such pollutants were not measured here and theoretically could contribute to some of the unexplained variation in the models. However, while potential effects of pesticides are largely unknown, other micropollutants are present and a potentially growing concern (Mahler & Musgrove, 2019), as is the case in many, if not most, aquatic ecosystems (Rajan et al., 2024).

The decline of insect populations has gathered much attention in recent years (Cardoso et al., 2020; Wagner et al., 2021), but direct assessment of freshwater insect populations has been limited and few prominent studies have indicated positive responses or no net change (Crossley et al., 2020; Haase et al., 2023; van Klink et al., 2020). Detailed species-level analyses of temporal trends are lacking among aquatic insects, and many studies that do report trends often lack robust datasets (e.g., Jenderedjian et al., 2012; Nakanishi et al., 2020; Tsyrlin et al., 2022; Van Strien et al., 2024). While this study presents data on just two species, it illustrates the type of data and monitoring programmes needed to understand even at a basic level if populations are changing and what factors are potentially causing those changes.

This study presents an important example of an endangered aquatic insect species that has a biological monitoring programme and long-term data covering the majority of the species' distribution, and we illustrate the challenges of studying both spring-endemic species and insect populations more broadly. Within this spring system, there were challenges with finding environmental variables that covary with CSRB abundance, ultimately leaving spring flow as the primary covariate relating to CSRB abundance. It is common in many systems to measure a standard suite of variables, such as the water quality variables we measured here, but it can be difficult to find factors affecting a species, and often various combinations of factors can be found to affect a species equally well (Araújo & Guisan, 2006; Graham, 2003; Murray & Conner, 2009). Therefore, careful consideration should be given to what is measured and why those measurements are being taken to appropriately inform and efficiently monitor a given species. Similarly, methods initially tested for studying this species were not well-standardized or particularly effective for monitoring (e.g., Barr, 1993; Bowles et al., 2003). Now, the cotton lure methodology has proven to be an effective method for monitoring adults of both of these elmid species. Although larvae did occur on cotton lures and the models suggest they can be monitored in this manner, their abundances were consistently lower than those of adults and not different across localities, suggesting that cotton lures are not as effective for monitoring larvae as they are for adults. Nevertheless, the data from adults on cotton lures demonstrate that it often requires taxon- and/or system-specific methodology to effectively monitor a species. Monitoring programmes themselves are important components of threatened species conservation, but appropriate monitoring techniques, data collection and use of the data are critical for ensuring that monitoring leads to effective protection efforts (Robinson et al., 2018).

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Aquatic insects have long received little conservation attention, even though freshwater habitats may be at higher risk of environmental change than terrestrial habitats and the taxa that are often the focus of freshwater conservation efforts (particularly fish) both prey on and have predation-risk effects on insects (Dudgeon et al., 2006; Pope et al., 2009; Resetarits et al., 2021). Here, we presented the case of a beetle that, by being listed as endangered, has served as a driver behind the restoration and conservation of its habitat and that of several other endemic species. Actions taken to preserve spring flow and water quality in the aquifer should not only help protect the CSRB, but also the many other spring- and aquifer-endemic species of central Texas. Twenty-one years of monitoring and over a decade of additional research have led to a greater understanding of the CSRB than of most other small aquatic beetle species. Yet, at present, average abundances sit at the lowest levels since monitoring began. Clearly, there is more to learn about this species to improve its conservation and support its recovery.

AUTHOR CONTRIBUTIONS

Matthew R. Pintar: Investigation; writing – original draft; methodology; visualization; writing – review and editing; formal analysis; project administration. **Kyle T. Sullivan:** Investigation; writing – original draft; methodology; visualization; writing – review and editing; formal analysis.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the study are available in Dryad at https://doi.org/10.5061/dryad.gmsbcc34c (Pintar & Sullivan, 2026).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Figure S1. Example of an upwelling site with a sandy substrate in the Spring Island area (site SI-23-03).

Figure S2. Example of an upwelling site in rockier substrates in the Spring Island area (site SI-OG-03).

Figure S3. Example of a larger spring site in Spring Run 3 (site SPR3-OG-04) that during most sample periods had lower water levels than shown here in January.

Figure S4. Example of a terrestrial margin site along the Western Shoreline (site WS-OG-10).

Figure S5. Daily total system spring flow from Comal Springs from the start of 2023 through spring 2024 with grey-shaded areas representing the five sampling periods (sampling periods 1–5; Table S1).

Figure S6. Photograph of one lure after retrieval exhibiting black, yellow orange and a small patch of purple biofilm (often blends in with the black).

Table S1. List of sampling periods with the start and end dates (earliest date lures were set, latest date lures were retrieved) and the mean 30-day total system spring flow over each sampling period.

Figure S7. Map of the 83 sample locations (red dots) throughout the Comal Springs with CSRB occupied habitat (grey area; areas where the CSRB have been recorded in the past 10 years).

Figure S8. Map of sectors (spatial groupings of sites) at Spring Island.

Figure S9. Map of sectors (spatial groupings of sites) at Spring Runs 1 and 3.

Figure S10. Map of sectors (spatial groupings of sites) at the Western Shoreline.

Table S2. Comparison of alternative models X = factor included in model; XX = factor included in model and significant ($p < 0.05$); blank = factor excluded from model.

Table S3. Total number of samples and larval and adult CSRB and MIPU found during the study, excluding all lures that were dry or disturbed.

Figure S11. Mean (±SE) of the natural log-transformed number of CSRB adults in the three primary sampling localities (excluding Spring Run 1) over the course of the study (by sampling period).

Figure S12. Mean (±SE) of the natural log-transformed number of CSRB larvae in the three primary sampling localities over the course of the study (by sampling period).

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Figure S13. Mean (±SE) of the natural log-transformed number of MIPU adults in the three primary sampling localities over the course of the study (by sampling period).

Figure S14. Mean (±SE) of the natural log-transformed number of MIPU larvae in the three primary sampling localities over the course of the study (by sampling period).

Figure S15. Scatterplot of 51 measurements for which there were measurements of both flow (cm/s) measured with the Hach flow meter and flux (mL/cm^2/s) measured with the seepage meter at upwelling sites. This relationship was used to convert flux measurements into approximate flow measurements for direct comparison of the two methods.

Figure S16. Boxplots of dissolved oxygen by locality and sampling period when lures were set.

Figure S17. Boxplots of conductivity by locality and sampling period when lures were set.

Figure S18. Boxplots of temperature by locality and sampling period when lures were set.

Figure S19. Boxplots of lure depth by locality when lures were set.

Figure S20. Boxplots of average substrate size by locality when lures were set. Points near the same *y* value generally are from the same site across different sampling periods.

Figure S21. Boxplots of average site-level spring flow (mean of starting and ending measurements) grouped by locality and sampling period.

Figure S22. Mean (±SE) of CSRB adult and larval abundance by locality (excluding Spring Run 1) based on the presence/absence of wood, leaves, roots or the three combined (all organic material).

Figure S23. Boxplots of total biofilm coverage on lures by locality and sampling period when lures were retrieved.

Figure S24. Locations of stations used to measure local spring flow discharge at Comal Springs. Cross section spring flow measurements were conducted using a HACH FH90 flowmeter and an adjustable wading rod. Spring flow at M9 locations were measured with an acoustic Doppler device used by the Edwards Aquifer Authority.

Table S4. Spring flow discharge stations used to assess local spring flow effects on CSRB relative abundance at each sector per site (see Appendix Figs. S7–S10 for sectors).

Table S5. Summary of parameter estimates, standard errors (SE) and performance metrics for the linear mixed-effects model used to predict station-level discharge.

Table S6. Summary statistics for 30-day local spring flow average (cfs) during the study duration (April 2023–May 2024) for discharge stations linked to each site.

Figure S25. Fitted predictions of discharge (cfs) as a function of mean daily total system spring flow (cfs) across the eight stations in Comal Springs. Solid lines and grey polygons represent the line of best fit and ±1 standard error, respectively.

Figure S26. Local spring flow time series from 2001 to 2024 at eight stations discharge measurement stations in Comal Springs. Solid lines represent LMM predictions per station and grey data points denote observed discharge measurements from 2003 to 2024.

Table S7. Estimates (90% Bayesian credible interval) of performance statistics used to evaluate the goodness of fit for generalized linear mixed-effects models of relative abundance.

Figure S27. Relative abundance (A) and apparent occupancy probability (B) relationships with the 30-day local spring flow average among sites for CSRB adults and larvae. Solid lines represent mean relative abundance predictions by the posterior distributions of each parameter and solid polygons denote 90% Bayesian credible intervals.

Figure S28. Relative abundance (A) and apparent occupancy probability (B) relationships with the total biofilm average among sites for CSRB adults and larvae. Solid lines represent mean relative abundance predictions by the posterior distributions of each parameter and solid polygons denote 90% Bayesian credible intervals.

Figure S29. Relationships between random effects, thin-plate spline smooth terms and CSRB abundance. 'SiteID2' is each spring site; 'Sample.project2' is the project during which data were collected; 'Method2' is the method of collection (cotton lure or preceding cotton cloth method); '30Day_SF_Avg' is the average spring flow calculated for each locality over the 30 days prior to each observation.

Figure S30. Relationships between random effects, thin-plate spline smooth terms and *Microcylloepus pusillus* abundance. 'SiteID2' is each spring site; 'Sample.project2' is the project during which data were collected; 'Method2' is the method of collection (cotton lure or preceding cotton cloth method); '30Day_SF_Avg' is the average spring flow calculated for each locality over the 30 days prior to each observation.

Figure S31. A schematic of the proposed conceptual framework illustrating how generalized linear mixed-effects models can be used as a tool for assessing Comal Springs Riffle Beetle populations under the EAHCP Biological Monitoring program.

Figure S32. Diagnostic plots for residuals of the generalized linear mixed-effects model used to forecast CSRB adult relative abundance during the fall 2024 EAHCP biological monitoring event. Plots include a histogram of residuals (A) and scatterplots of residuals versus predictions (B), the 30-day local spring flow average (C) and total biofilm (D). The black lines fit to the scatterplots on panels (B–D) represent quantile regression lines of best fit (quantile = 0.5).

Table S8. Summary of observed and expected (90% BCI) median relative abundance of CSRB adults during the fall 2024 EAHCP biological monitoring event.

Figure S33. Boxplots comparing CSRB adult relative abundances observed in fall 2024 versus expected values predicted by the generalized linear mixed-effects model. The thick horizontal line in each box is the median, and the upper and lower bounds of each box represent the interquartile range. Whiskers represent minimum and maximum values up to 1.5 times the interquartile range.

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