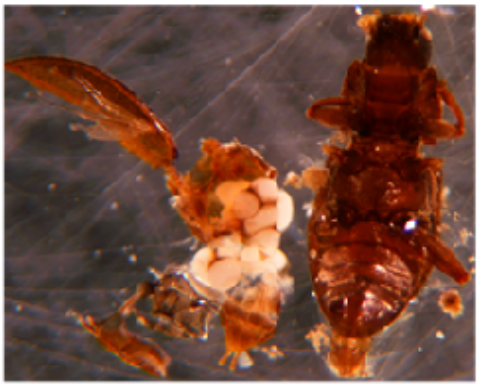
Evaluation of the Life History of the Comal Springs Riffle Beetle

Egg



to

Adult

Photographs by Randy Gibson and Mike Quinn

LITERATURE REVIEW and YEAR ONE METHODOLOGY

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February 26, 2016

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BACKGROUND

Riffle beetles (Family Elmidae) are small aquatic beetles that occupy larger substrates in swift habitats of high quality, low temperature, streams and rivers. They respire through a plastron (Brown 1987, White and Roughley 2008, Elliott 2008a) and are typically sensitive to pollutants and environmental change. The Comal Springs riffle beetle (*Heterelmis comalensis*) was Federally listed as endangered in 1997 due to threats caused by potential decreases in spring discharge attributed to drought or excessive groundwater extraction. It is also vulnerable to groundwater contamination from urban runoff, agricultural waste and toxicants, and leaking storage facilities and pipelines. *Heterelmis comalensis* are flightless, non-vagile, and associated with gravel substrates near spring sources. Adults are approximately 2 mm long, females are slightly larger than males, and it is thought that they feed on decaying organic matter and awfuchs.

Habitat Conservation Plan (HCP) applied research conducted over the first three years of the program has demonstrated that aquatic vegetation as fountain darter habitat, and fountain darters themselves are quite tolerant to environmental changes tested thus far. This finding suggests that *H. comalensis* may in fact be a more appropriate sentinel species for the Comal system. This is extremely important in that the adopted HCP flow regime exhibits periods of extended drying of the spring runs, and areas along the western shoreline and Spring Island (these areas are the presumed strong hold for the riffle beetle in the Comal system). Although the HCP flow regime is not projected to be as severe as experienced in the drought of record (DOR) relative to minimum flows, it does project extended periods of < 100 cfs which are beyond what was observed during the DOR. With *H. comalensis* potentially serving as a sentinel species for the Comal system, understanding their life history, tolerance and surface/subsurface interactions is vital to making sound HCP Phase II decisions.

As with most rare species, conservation of *H. comalensis* requires strategies to maintain aquatic habitats that provide for growth, reproduction, and population viability. These strategies are most effectively developed by determining habitat preferences (e.g., water temperature, depth, current velocity, etc.) of the species, or by experimentally examining the effects of different environmental conditions on body condition. Information from these types of studies can provide insight into the importance of a variety of habitat metrics, and the range in habitat conditions hospitable to the livelihood of *H. comalensis*. The limited distribution, sensitivity of habitats, small body size, and difficulties involved in sampling the three dimensional habitat of *H. comalensis* have proven to make determining habitat preferences through field studies problematic; further emphasizing the importance of laboratory investigations as proposed herein.

This HCP applied research strives to evaluate the life history and developmental stages through the life cycle of *H. comalensis*. Thus far, the life cycle has been documented to take more than a year in the laboratory (Randy Gibson, pers. comm.); therefore a two year study was proposed and approved to investigate important life history questions. A phased approach is proposed starting with Year 1 (2016) studying factors contributing to production of eggs and larval development and Year 2 (2017) building off the knowledge learned to further explore and achieve pupation and complete the full life cycle. Accompanying both years of life cycle studies will be a concurrent investigation intended to assess the factors that contribute to riffle beetle condition and health.

LITERATURE REVIEW

Population Distributions and Habitat Associations of the Comal Springs Riffle Beetle

Heterelmis comalensis is a crenophilic riffle beetle that is endemic to eucrenal habitats of Comal Springs, Comal County and San Marcos Springs, Hays County, both in Texas. The niche of *H. comalensis* in the Comal system was determined to be highly restricted spatially to within approximately 80 cm from spring openings throughout the primary and smaller spring outlets along the spring runs (Cooke 2012). *H. comalensis* is found at the depths of 2-10 cm in hard-packed gravel habitats commonly found adjacent to spring openings (Bosse 1979). The precise mechanisms for the apparent spatial restriction of *H. comalensis* within the Comal system are unknown, but may involve spatial variation in water quality parameters and microhabitat associations. Data indicate that riffle beetles prefer spring water characterized by high CO_2, low dissolved oxygen (DO), and slightly lower pH in comparison to surface-water dominated streams (Cooke 2012). Other species of more widespread elmids (i.e., *Stenelmis* spp.) also exhibit habitat preferences for stable gravel-cobble substrates and coarse woody debris, rather than unstable sand and mud substrates (Phillips 1995). It is currently thought that a reduction in spring flow that leads to loss (desiccation) of habitat or reduces water quality of occupied riffle beetle habitat will likely impact their fitness and survival. Undoubtedly, water quality will be the primary issue in the spring runs and along the western shoreline during substantial low-flow events as springs within these areas cease flowing and the habitat associated with the presence of the Comal Springs riffle beetle dries. However, as flows decline at Comal Springs and the remaining aquatic habitat is reduced to portions of Landa Lake along the western shoreline downstream of Spring Island (EARIP 2012), it is likely that the water temperature will increase and DO concentrations will drop. A recent study conducted for the EAHCP found that Comal Springs riffle beetles could tolerate rapid changes in temperature and DO concentrations (i.e., beetles could withstand up to 45°C before loss of function and tolerate 0 mg DO/L for several minutes without suffering obvious ill effects), but that their tolerance ranges to short-term temperature changes were substantially narrower than a closely-related elmid species (*Heterelmis glabra*; Nowlin et al. 2014). However, the sensitivity of riffle beetles to longer-term and more slowly-occurring changes in temperature and DO remain to be determined.

Dispersal Ability, Habitat Connectivity, and Life History of the Comal Springs Riffle Beetle

Although many adult aquatic coleopterans emerge from aquatic habitats for dispersal flights to other aquatic locations, many riffle beetle species typically cannot disperse great distances via flight (White and Roughley 2008). Indeed, some elmid species are thought to be flightless as adults (Elliott 2008a) and other species can fly relatively short distances after emergence but their flight muscles degenerate after they re-enter the water (Hinton 1976). The complete loss of adult flight (degenerated wings) or the rapid loss of wings after adult emergence suggests that retaining flight capability may not be compatible with utilization of plastron respiration because of the maintenance of substantial sub-elytral air space required for functional wings (Thorpe and Crisp 1947). Thus, smaller bodied adult elmids like *H. comalensis* typically do not have the ability to disperse great distances and move relatively slowly via crawling in their aquatic habitats or through drifting downstream. On benthic surfaces, smaller-bodied invertebrates like elmids are more resistaned to dislodging during high flow events than larger-bodied invertebrates (Turcotte and Harper 1982), but the use of benthic surface habitats can vary substantially among species and within life stages of a single species. In general, elmid larvae are less sensitive to lower water velocities and hydraulic stagnation than adult stages (Walters and Post 2011) because larvae are less dependent on flowing water conditions (i.e., less rheophilous) than adults because they utilize

gills for respiration (Elliott 2008). Later larval elmid instars can develop tracheal air sacs that provide a Cartesian driver system for controlling the specific gravity of the body and allow drift towards preferred pupation sites along stream banks (Brown 1987). Drifting of individuals occurs mostly at night, most likely in response to gaining access to food resources or to escape sub-optimal environmental conditions (Elliott 2008b; Brown 1987; Reisen 1977). Higher mortality rates of newly hatched or overwintering larvae occurs during drift events, as they are then at high risk of being washed away by the current and/or dispersed to sub-optimal habitats (Reisen 1977; Elliott 2008a).

Variation in environmental conditions can alter the timing and magnitude of emergence of adult elmids, including *Microcylloepus pusillus* and *Heterelmis* spp. (Reisen 1977). In temperate systems with a high level of seasonal variation in environmental conditions (e.g., Quebec, North America), individual elmids can persist in the larval stage for 2-3 years and the adult stage lasts for approximately a year (Lesage and Harper 1976). In temperate systems, both adult and larval elmids exhibit a great deal of seasonal variation in life stage timing, and air/water temperatures greatly influences the duration of the pupation period (Lesage and Harper 1976). In contrast, *H. comalensis* and *M. pusillus* in the Comal Springs system exhibit non-seasonally influenced emergence patterns and have overlapping, asynchronous generations (Bowles et al. 2003). This lack of seasonality in emergence and life history patterns in *H. comalensis* is largely thought to be a consequence of environmental conditions at spring-influenced systems like Comal and San Marcos because they exhibit little seasonal variation. In other systems with limited variation in environmental conditions (e.g., the tropics), emergence of *Heterelmis* adults occurs during periods of low current velocities and high food availability, and oviposition occurs when temperature is highest and water level is lowest (Passos et al. 2003). It is important to note that although the USFWS can successfully house both adult and larval *H. comalensis* to the extent that adults mate and oviposit in aquaria, they have so far had only minimal success in getting adults to emerge after pupation. Currently, there is a clear need to better understand potential mechanisms and conditions leading to successful adult emergence of *H. comalensis*.

Surface-Subsurface Interactions for Flowing Water Invertebrates

The subsurface hyporheos, or hyporheic zone, of flowing water systems may act refuge for benthic invertebrates during periods of low flow or even in apparently dry stream beds (Williams and Hynes 1974). It provides refuge from drying and enables invertebrates to recolonize the surface once the disturbance has passed (Dole-Olivier et al. 1997). Drought-induced changes to in-stream environment such as hydraulic stagnation, increased/decreased water temperatures, increased fine sediment deposition, and altered macrophyte composition can affect the macroinvertebrate community by reducing habitat quantity and quality as well as access to preferred food resources. During low flow periods, the contraction of wetted stream width can cause in-stream organismal densities to increase and lead increased resource competition in the hyporheic zone (Dewson et.al 2007). Complete cessation of flow in the hyporheic zone can lead to loss of suitable habitat for invertebrates seeking refuge in the hyporheos through the eventual complete desiccation of hyporheic sediments (Boulton and Stanley 1995), anoxia in the hyporheos (Smock et al. 1994), and/or the lack of interstitial habitat due to clogging of interstices by fine sediments (Bo et al. 2006). In is also unknown whether a reduction in spring flow may lead to the disconnection of *H. comalensis* from potential or preferred food sources, such as terrestrial organic matter and detritus which may be most concentrated along the bank.
In addition to its limited geographic distribution, specificity in preferred habitat types, lack of mobility, and potential sensitivity to habitat degradation, the genetic variation of *H. comalensis* populations across the Western Shoreline, Spring Island, and San Marcos springs populations suggests limited gene flow among these populations. Therefore, if springs at Comal or San Marcos springs cease to flow for

extended periods of time, genetic variation among the remaining populations could be lost (Gonzales 2008).

Although *H. comalensis* was described as a species after Comal springs stopped flowing during the drought of record (Bosse et al. 1988), it has been assumed that *H. comalensis* populations were present in the Comal system prior to the drought of record and were able to persist for the 144-day no-flow period in 1956. It is remains unknown precisely how beetles persisted in the Comal system during the drought of record, how or if the drought of record affected riffle beetle populations, and if riffle beetles have the ability to rapidly recover from a large-magnitude drought events. It has been hypothesized that *H. comalensis* persisted through the drought of record through life cycle aestivation or by retreating into spring heads, the aquifer, or down into the hyporheos (Bowles et.al 2003). Previous research has determined that for perennial species of riffle beetles (i.e., species that live for multiple years), pool habitats connected via some flowing surface water can serve as refugia for both larvae and adults during drought periods (Burk and Kennedy 2013). Elmid use of these refuge areas is thought to be associated with the relatively higher water quality and constancy of flow in these habitat patches during drought conditions (Burk 2012). In addition, during extreme drought events, elmids can take refuge in shaded disconnected pools where environmental temperature and evaporative losses are moderated by riparian shading (Burk and Kennedy 2013). However, elmids may also utilize subsurface (hyporheic) environments when flows are low; elmid body shape is such that they can tolerate small spaces (elmid adults are typically of small body size, while larvae have slender, flexible bodies), and elmids have been found to survive in the hyporheos for relatively long periods of time during periods of low flow (Boulton and Foster 1998; Marchant, 1988).

Food Habits and Trophic Ecology of Comal Springs Riffle Beetle

Potential food resources for *H. comalensis* have not been clearly identified in the Comal and San Marcos systems. Most literature sources state that riffle beetles are generally biofilm scrapers that can utilize detrital materials (Brown 1987). Currently, the standard capture method for *H. comalensis* in Comal is through the use of cloth lures (R. Gibson, USFWS; *pers. comm.*). Presumably, Comal Springs riffle beetles are attracted to the lures to gain access to the biofilms that grow there. A more widely-distributed elmid species, *Heterelmis vulnerata*, is often associated with coarse woody debris with biofilm coverage and loose bark and/or interstitial spaces. The biofilm and interstitial spaces are thought to be used as concealment from the predators and biofilms may serve as algal and fungal food sources for the beetles (Phillips 1995). Seagle (1982) found that the gut contents of larvae and adults of three different riffle beetle species (*Stenelmis crenata, Stenelmis mera,* and *Optisoservus trivittatus*) was dominated by detritus-like materials, including wood xylem and unidentified organic matter and mineral particles, while algal material was consumed to a much lesser extent. Thus, it has been suggested that elmids should be reclassified as detritivorous herbivores rather than as strictly herbivores, with the exception of known xylophagus genera (i.e., *Lara*) (Seagle 1982). Cannibalistic foraging has been observed in some elmids (i.e., *M. pusillus*), but this behavior was attributed to nutritional deprivation and is probably not a common foraging strategy (Brown and Shoemake 1969). Currently, the precise food sources and trophic ecology of *H. comalensis* remains unknown.

Hypothesized life history

Despite no eggs having been observed in the wild or in captivity, dissected a female in captivity was found to be carrying around 10 relatively large eggs (Figure S1). No information exists on deposition sites, hatch time, and diapause for *H. comalensis* however it was speculated by Brown (1987) “that most

elmids glue their eggs singly or in small clusters to undersides of submerged rocks, wood, or plant stems, depending on habitat preference of the species." It remains to be seen if *H. comalensis* lay eggs in individually or in clutches and where females prefer to lay their eggs. Presumably *H. comalensis* eggs do not overwinter or display diapause (dormancy) as in other aquatic inverts with seasonal patterns because it has been shown that *H. comalensis* exhibit no seasonality in the wild (Bowles et al. 2003) or in captivity [San Marcos Aquatic Resource Center (SMARC) refugium data]. Riffle beetle eggs typically have short incubation times of 5-15 days depending on temperature (Brown 1987) which is expected to be the case in *H. comalensis*.

After hatching, it is presumed that *H. comalensis* larvae undergo several molts; molting once between instars. Other genera of elmids studied have 6-8 larval instars across a duration of 6-36 months with developmental rate varying with temperature and food availability (Brown 1987, White and Roughley 2008). Cooke (2012) estimated *H. comalensis* have approximately seven instars by measuring preserved larvae. Aside from this no other information on larval development is known. Like other elmids, *H. comalensis* larvae have gills and are aquatic, often inhabiting similar habitats as adults subsisting on microorganisms and debris scraped from substrate (Brown 1987). Later instar riffle beetle larvae develop tracheal air sacs which might aid in drifting to suitable pupation sites or escaping poor environmental conditions (Brown 1987). Typically, elmids pupate above the water line in moist soils, under rocks, or in rotting wood (Brown 1987, White and Roughley 2008). Above water pupation might help in establishing a functional plastron in the adult when shifting from gill respiration as a larva. However pupation in *H. comalensis* has been observed to take place under the surface of the water in captivity at the SMARC (Huston and Gibson 2015) and in the wild (pers. obs.). Currently, the requirements for pupation of *H. comalensis* are still unknown.

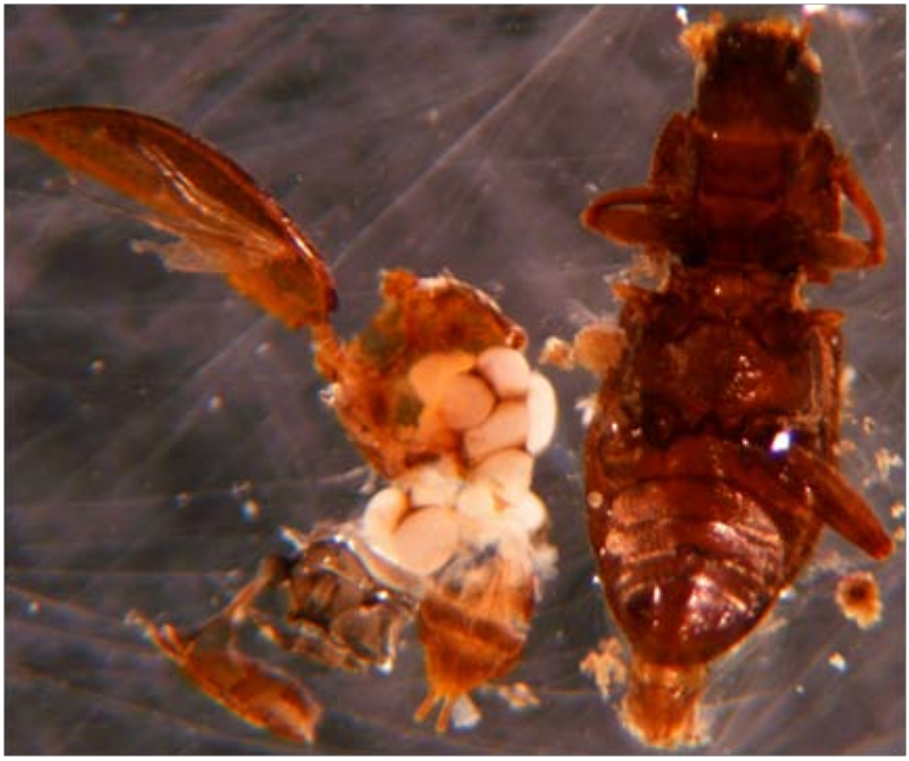

Figure S1. Photograph of a dissected single female in captivity that was found to be carrying around 10 relatively large eggs

As it is unknown how long it takes for larvae to maturate into pupae, it is unknown how long pupae must incubate before molting into adults. As it unknown how long beetles can live as adults, it is impossible to presently estimate the typical life span of *H. comalensis*. However, wild caught adults have been maintained in captivity at SMARC for up to 16 months and other species of elmids have survived for several years in captivity (Brown 1973, White and Roughley 2008) therefore suggesting the lifespan of *H. comalensis* is likely quite long.

METHODS

YEAR 1 (2016)

EGG PRODUCTION AND MATING

Subtask 1.1 – Collection of study organisms (February - March)

The first step in the process of producing eggs will be to obtain adult *H. comalensis* from Comal Springs. Adults will be collected using cotton lures (Gibson et al. 2008). Lures will be retrieved approximately two to three weeks after they are set in spring openings in Spring Run 3, Western Shoreline, and around Spring Island. Any larvae collected will be returned to the spring they were collected from and adults will be brought back alive to the SMARC where all phases of egg production experiment will be conducted. Once at SMARC, a subset of individuals (n=30) will be randomly selected from the collected individuals for use in Subtask 1.2. All other individuals will be placed in quarantine and held for at least two weeks prior to being used in any subsequent research.

Subtask 1.2 – Noninvasive/nonlethal method of sexing wild-caught adults (February-March 2016)

The proposed experimental design calls for the pairing of one male with one female to be held together to mate and then produce eggs. In order to produce pairs of males and females, a method needs to be developed to reliably sex individuals to insure that no same sex pairs are formed unintentionally. Unfortunately, no method exists to date that can reliably determine the sex of riffle beetles. Most methods that have been employed thus far require the euthanasia and subsequent dissection of individuals to determine sex; unfortunately these methods are not useful for sexing live individuals. However, it may be possible to develop a nonlethal method of sexing individuals. As mentioned herein, there appears to be a relationship between size and dimensions and sex in adult *H. comalensis* (Bosse et al. 1988). Therefore, it might be possible to sex individuals just on the basis of their external morphology. It may also be possible to anesthetize individuals using CO_2 and then extrude their genitalia. However, this methodology may be a bit more invasive than what would be preferred to insure than none of the test subjects used for mating trials were harmed.

Subtask 1.3 – Mating and egg production (March-June)

Once a reliable method is developed for sexing individuals without harming them, male-female pairs will be formed and one pair each will be placed into reusable Keurig filter cups suspended in a static array. Thirty-two male-female pairs will be formed in total. Each pair will be assigned a unique accession number and then randomly assigned to one of eight experimental groups with four replicates per experimental group. The eight experimental groups are as follows:

- Leaves
- Leaves + cotton cloth
- Leaves + cotton cloth + rock
- Cotton cloth + rock

- Cotton cloth
- Leaves + rock
- Rock
- Control (none of the above factors present)

Before assigning to experimental groups, detailed morphological data will be taken on both the male and female specimen of each treatment group. This information will be used to determine if any morphological factors contributed to the success of egg production. Pairs will be checked twice weekly for eggs. If eggs are observed they will be counted. Adults will then be removed from the Keurig cups and placed into a new cup with the same combination of treatment factors. If eggs continue to be produced this step will be repeated. Differences in the number of eggs that are produced on the each substrate combinations over a given period of time will be analyzed for significance via three-way analysis of variance (ANOVA).

Subtask 1.4 – Incubation of eggs

Once eggs are produced they will be monitored until they hatch by checking twice weekly. We will assess differences in the hatch rates and amount of time eggs incubated using a combination of regression analyses and one-way ANOVAs using the different treatment groups of the parents to determine which factors contribute to the greatest production of viable eggs. After hatching, a subset of 1st instar larvae will be preserved for condition analysis at Desert Research Institute (DRI); see Condition Index task below for explanation. The amount of larvae used for condition analysis will depend on the number of larvae produced; not to exceed 5% of total larvae.

Subtask 1.5 – Mating observations

Following findings from Subtask 1.3, further study of the nuances of *H. comalensis* mating will be studied. A subset of conditions tested in Subtask 1.3 found to produce the most eggs and therefore presumed to be the most "ideal" conditions for *H. comalensis* mating will be utilized in this phase of study. Four replicates of each type of substrate will be made in individual containers with one pair placed into each container. After establishing mating trial replicates, overhead video data or repeated interval photography will be taken on all pairs for a duration of 4 to 5 months. Daily observations will also be made and notes will be documented if pairs are observed in amplexus. The mean duration of mating events, the number of mating events, and the frequency of matings will be compared across substrate types *via* ANOVA.

LARVAL DEVELOPMENT

Complete development into adults from small larvae has been observed to take place as fast as 4 months at SMARC, however single larvae have been held as along as at 1.5 years at SMARC without development into larger instars. This might be due to nutritional requirements of *H. comalensis*. Because of this, various sources of nutrition will be tested on the development of the larvae and this study will likely take more than a year to complete. Because this will take many holding containers and extensive amount of time and labor to complete, these studies will take place at both the Freeman Aquatic Biology Building (FAB) and SMARC in order to maximize space, resources, and provide replication of data as well as a backup in case of equipment failure. General design for this task is as follows:

Subtask 2.1 – Individual holding - development

Newly hatched 1st instar larvae will each be given a unique accession number and individually assigned randomly to one of eight experimental groups with a combination of factors the same as those listed in Subtask 1.3. Again, these experimental groups are as follows:

- Leaves
- Leaves + cotton cloth
- Leaves + cotton cloth + rock
- Cotton cloth + rock
- Cotton cloth
- Leaves + rock
- Rock
- Control (none of the above factors present)

1st instar larvae will then be placed into individual holding chambers (reusable Keurig filter cups) suspended in a static array with flowing Edwards Aquifer water. Chambers will then receive the assigned treatment substrate. Experimental replication will depend on the availability of larvae produced but will be evenly spread across experimental groups. Approximately one half of the experimental replicates will be randomly selected to remain at SMARC while the other half will be moved to the FAB on Texas State University campus. Larvae will have development continuously observed and monitored during 2016 and 2017 by checking twice monthly for growth and/or molts. To do this, larvae will be anesthetized and have total body length and head capsule width measured using Olympus Cellcens camera system and measuring tool software at the standard shutter speed of 3.395 milliseconds. Growth and molt rate will be analyzed for significance *via* univariate analyses (e.g., ANOVA). Throughout this process of development, portions of every instar of larvae will be preserved for condition analysis at DRI; not to exceed approximately 2% of available larvae.

Subtask 2.2 – Group holding - habitat preference

H. comalensis in culture at SMARC readily produce numerous larvae. Therefore, at any given time there are usually hundreds of larvae of various instars in culture at SMARC. Because these larvae are of unknown age and instar, they are not useful for the study of larval development. However, these larvae are useful for the study of larval physiological tolerances and habitat preferenda. To study habitat preferenda, larvae will be taken from refuge culture tanks and placed into tanks sectioned with various substrates. Four tanks will be sectioned into eight sections of equal area. Each tank will have one section for each of the following substrate types:

- Leaves
- Leaves + cotton cloth
- Leaves + cotton cloth + rock
- Cotton cloth + rock
- Cotton cloth
- Leaves + rock
- Rock
- Control (none of the above factors present)

The arrangement of the different sections will be randomized for each tank in an effort to ensure that artifacts of the distribution of the different substrate types does not confound results. Sections will be sectioned off in a way that allows for the passage of larvae so that larvae can freely move about between them. Each tank will be set up with a flow through system of Edwards Aquifer water and calibrated to

receive approximately equal flow. Once sectioned, 80 larvae taken from refuge culture tanks will be placed into each habitat preferenda tank. Ten larvae will be randomly assigned to each section of each tank to start experimental trials. Afterwards, each section will be examined monthly for larvae. Larvae in each section will be counted, have length and head capsule width measured using Olympus Cellcens camera system and measuring tool software at the standard shutter speed of 3.395 milliseconds and then returned to the section they were found in. If any pupation or adults are observed, the substrate type they were found on will be noted. We will assess differences among substrate types in terms of the number of larvae in each section using ANOVA. In addition substrate preferences and shift in preference exhibited by different developmental stages will be assessed *via* a combination of univariate (Students t-test) and multivariate analyses (MANOVA).

CONDITION INDEX

Dr. Sada manages a laboratory at the Desert Research Institute (DRI) that focuses on the ecology of benthic macroinvertebrates (BMIs) in springs, streams, and rivers. His studies in the Walker River (western Nevada) examined relationships between BMIs and the environment to guide restoration (e.g., Hogle et al. 2014), and influences of the environment on secondary production (e.g., Mehler et al. 2014). Generally larger females produce more, and larger eggs, than small females, and larger males are more reproductively fit than small males. Secondary production in the Walker River was estimated by examining mayfly (genus *Baetis*) length-mass relationships and calculating the annual change in biomass per unit of area. These relationships were then examined in context of river environments to determine conditions where secondary production was highest and lowest.

The purpose of the riffle beetle condition index task is to first determine analytical methods and to assess relationships between environments and habitat associations on *H. comalensis* body condition. We hypothesize that the length-mass relationship of *H. comalensis* will be indicative of body condition, whereby the condition of slender individuals will be lower than more robust animals. Hence, environments producing slender individuals will be considered less suitable to *H. comalensis* than conditions that produce more robust animals.

Adult, juvenile, pupae, and potentially eggs will be provided to Dr. Sada's laboratory at DRI from captive *H. comalensis* populations. The focus of Year 1 will be to begin developing sampling and analytical methods. It is anticipated that material will be frozen and sent to DRI on dry ice to measure size (length, volume, etc.) and biomass. It is anticipated that size will be measured using a compound microscope and biomass measured as ash free dry weight. Important factors to be determined include: 1) can surrogate species of riffle beetles (e.g., widespread taxa such as *Microcylloepus* spp.) be used in studies to determine sample and analytical methods, 2) are individuals sufficiently large to analyze separately, or is the biomass of several individuals required to meet the minimum requirements to measure biomass in terms of hundredths of milligrams, 3) if several individuals are required, how many, and 4) what statistical analyses will most effectively reveal 'healthy' vs. 'diminished' body condition.

YEAR 2

As referenced above, it is anticipated that the second year of life history studies will focus on larval completion/pupation, adult life span and fecundity, and a continuation of the condition index evaluation initiated in the first year. It is envisioned that upwelling substrate / water line trials and duration / flow requirement studies may be conducted to investigate larval completion and pupation. Initial condition index development insight obtained during Year 1 will be referenced to establish Year 2 methodologies.

If condition index development is successful, it is anticipated that examinations of adult, larval, pupae, and eggs produced in a variety of environmental conditions will be evaluated in Year 2. In addition to condition assessments, life stage samples may be examined to determine if salient morphological characteristics differ among individuals from different environments.

For all study components, the methodologies for Year 2 will build upon the insight obtained the first year and be presented to the HCP Science Committee for review and comment later in 2016.

LITERATURE CITED AND REVIEWED

BIO-WEST. 2014. Effect of low-flow on riffle beetle survival in laboratory conditions. Edwards Aquifer Authority study no. 13-658-HCP. 34 pp.

BIO-WEST. 2015. Comal Springs riffle habitat connectivity study. Edwards Aquifer Authority study no. 14-729-HCP. 57 pp.

Bosse, L.S. 1979. A survey of the adult Dryopoids (Coleoptera) in the San Marcos and Comal Rivers in central Texas [thesis]. [San Marcos (TX)]: Southwest Texas State University.

Bo, T., Cucco, M., Fenoglio, S. and Malacarne, G. 2006. Colonisation patterns and vertical movements of stream invertebrates in the interstitial zone: a case study in the Apennines, NW Italy. Hydrobiologia 568: 1573-5117.

Boulton, A. J. and Stanley, E. H. 1995. Hyporheic processes during flooding and drying in a Sonoran Desert stream. II. Faunal dynamics. Archiv für Hydrobiologie 134: 27-52.

Bosse, L.S., Tuff, D.W. and Brown, H.P. 1988. A new species of Heterelmis from Texas (Coleoptera: Elmidae). Southwestern Naturalist 33:199-203.

Boulton, A.J. and Foster, J.G. 1998. Effects of buried leaf litter and vertical hydrologic exchange on hyporheic water chemistry and fauna in a gravel-bed river in northern New South Wales, Australia. Freshwater Biology, 40, 229–243.

Bowles, D.E., Barr, C.B. and Stanford, R. 2003. Habitat and phenology of the endangered riffle beetle *Heterelmis comalensis* and a coexisting species, *Microcylloepus pusillus*, (Coleoptera: Elmidae) at Comal Springs, Texas, USA. Archiv für Hydrobiologie. 156:361-384.

Brown, H.P., Shoemake, C. M. 1969. Cannibalism by a "herbivore," *Microcylloepus pusillus* (Coleoptera: Elmidae Proc. Okla. Acad. Sci. 48: 15.

Brown, H. P. 1973. Survival records for elmid beetles with notes on laboratory rearing of various dryopoids (Coleoptera). Entomological News 84:278-284.

Brown, H.P. 1987. Biology of riffle beetles. Annual Review of Entomology 32, 253-273.

Brune, G. 1981. Springs of Texas. Branch-Smith, Inc., Fort Worth, Texas.

Burk, R.A. 2012. Ecology and recolonization of benthic macroinvertebrates in groundwater dependent stream in North-central Texas during a supra-seasonal drought [dissertation]. [Denton (TX)]: University of North Texas.

Burk, R.A. and Kennedy, J.H. 2013. Invertebrate communities of groundwater-dependent refugia with varying hydrology and riparian cover during a supra-seasonal drought. Journal of Freshwater Ecology: 28(2): 251-270.

Cooke, M. 2012. Natural history studies on the Comal Springs riffle beetle (*Heterelmis comalensis*) [master's thesis]. [San Marcos (TX)]: Texas State University.

Dewson, Z.S, Alexander, J. and Death, R.G. 2007. A review of the consequences of decreased flow for instream habitat and macroinvertebrates. Journal of the North American Benthological Society. 26:401–415.

Dole-Olivier, M.-J., Marmonier, P., and Beffy, J.L. 1997. Response of invertebrates to lotic disturbance: is the hyporheic zone a patchy refugium? Freshwater Biology, 37: 257-276.

Edwards Aquifer Recovery and Implementation Program (EARIP). 2012. Edwards Aquifer Recovery and Implementation Program Habitat Conservation Plan. 414 p.

Elliott, J.M. 2008a. The ecology of riffle beetles (Coleoptera: Elmidae). Freshwater Reviews 1: 189–203.

Elliott, J.M. 2008b. Ontogenetic shifts in drift periodicity and benthic dispersal in elmid beetles. Freshwater Biology 53, 698-713.

Gonzales, T.K. 2008. Conservation Genetics of the Comal Springs Riffle Beetle (*Heterelmis comalensis*) Populations in Central Texas, with examination of molecular and morphological variation in *Heterelmis sp*. throughout Texas [master's thesis]. [San Marcos (TX)]: Texas State University.

Hardy, T.B, Kollaus, K. and Tower, K. 2010. Evaluation of the proposed Edwards Aquifer Recovery Implementation Program drought of record minimum flow regimes in the Comal and San Marcos River systems. Prepared for Edwards Aquifer Recovery Implementation Program. 80 p.

Hogle, C., D.W. Sada, and C. Rosamond. 2014. Using benthic indicator species and community gradients to optimize restoration in the arid, endorheic Walker River watershed, western U.S. River Research and Applications. DOI: 10.1002/rra.2765.

Hinton, H. E. 1976. Plastron respiration in bugs and beetles. Journal of Insect Physiology 22:1529-1550.

Huston and Gibson. 2015. Underwater pupation in the Comal Springs riffle beetle *Heterelmis comalensis* (Coleoptera: Elmidae). The Coleopterists Bulletin 69: 521-524.Coleopterist Bulletin.

LBG Guyton and Associates. 2004. Evaluation of augmentation methodologies in support of *in-situ* refugia at Comal and San Marcos Springs, TX, prepared for the Edwards Aquifer Authority. 192 p.

LeSage, L. and Harper, P.P. 1976. Cycles biologiques d'Elmidae (coléoptères) De ruisseaux des laurentides, Québec. Annales de Limnologie 12, 139-174.

Marchant R. 1988. Vertical distribution of benthic invertebrates in the bed of the Thomson River, Victoria. Australian Journal of Marine and Freshwater Research, 39, 775–784.

Mehler, K., K. Acharya, D.W. Sada, and Z. Yu. 2013. Environmental stoichiometry of basal resources and benthic macroinvertebrates along a land use gradient in a Great Basin watershed. Hydrobiologia DOI 10.1007/s10750-013-1549-y.

Mehler, K., K. Acharya, D.W. Sada, and Z. Yu. 2014. Factors affecting spatiotemporal benthic macroinvertebrate diversity and secondary production in a semi-arid watershed. Journal of Freshwater Ecology. http://dx.doi.org/10.1080/02705060.2014.974225.

Norris, C. W. 2002. Effects of variable flows on invertebrate drift in Comal Springs, Texas. Unpublished M.S. thesis, Southwest Texas State University, San Marcos.

Nowlin, W.H., Schwartz, B., Hardy, T. and Gibson, R. 2014. Determination of limitations of Comal Springs riffle beetle plastron use during low-flow study. Edwards Aquifer Authority study no. 14-14-697-HCP.

Palmer, R.A. 1994. Fluctuating asymmetry analysis: a primer. In, T.A. Markow (ed.). Developmental Instability: Its Origins and Evolutionary Implications. Kluwer Academic Press. London.

Passos, M.I.S., Nessimian, J.L. and Dorvillé, L.F.M. 2003. Life strategies in an elmid (Insecta: Coleoptera: Elmidae) community from a first order stream in the Atlantic Forest, southeastern Brazil. Acta Limnologica Brasiliensia, vol. 15, p. 29-36.

Phillips, E.C. 1995. Associations of aquatic Coleoptera with coarse woody debris in Ozark streams, Arkansas. The Coleopterists Bulletin, 49 (2):119–126.

Reisen, W.K. 1977. The ecology of Honey Creek, Oklahoma: downstream drift of three species of aquatic dryopoid beetle (Coleoptera: Dryopoidea). Entomological News 88, 185-191.

Seagle, H.H. 1982. Comparison of the food habits of three species of riffle beetles, *Stenelmis crenata, Stenelmis mera,* and *Optioservus trivittatus* (Coleoptera: Dryopoidea: Elmidae). Freshwater Invertebrate Biology 1, 33-38.

Smock, L.A., Smith, L.C., Jones Jr, J.B., and Hooper, S.M. 1994. Effects of drought and hurricane on a coastal headwater stream. Archiv für Hydrobiologie 131: 25-38.

Thorpe, W.H., and Crisp, D.J. 1947. Studies on plastron respiration. Part I. The biology of *Aphelocheirus* [Hemiptera, Aphelocheiridae (Naucoridae)] and the mechanism of plastron retention. Journal of Experimental Biology 24: 227–269.

Turcotte, P., Harper, P.P. 1982. The macroinvertebrate fauna of a small Andean stream, South America. Freshwater Biol. 12(5):41 1-20.

United States Fish and Wildlife Service (USFWS). 2007. Endangered and threatened wildlife and plants; Designation of Critical Habitat for the Peck's Cave Amphipod, Comal Springs Dryopid Beetle, and Comal Springs Riffle Beetle; Final Rule. Federal Register 72 (17 July 2007): 39248-39283.

Walters, A.W, Post, D.M. 2011. How low can you go? Impacts of a low-flow disturbance on aquatic insect communities. Ecological Applications. 21:163–174.

White, D. S., and Roughley, R. E. 2008. Aquatic Coleoptera - Elmidae (Riffle Beetles). Pages 632 in R. White, D. S., and R. E. Roughley. 2008. Aquatic Coleoptera - Elmidae (Riffle Beetles). Pages 632 in R. W. Merritt, K. W. Cummins, and M. B. Berg, editors. An Introduction to the Aquatic Insects of North America, Fourth Edition. Kendall/Hunt Publishing Company, Dubuque, Iowa.

Williams, D.D. and Hynes, H.B.N. 1974. The occurrence of benthos deep in the substratum of a stream. Freshwater Biology 4: 233-256.