EVALUATION OF THE LONG-TERM, ELEVATED TEMPERATURE AND LOW DISSOLVED OXYGEN TOLERANCES OF THE COMAL SPRINGS RIFFLE BEETLE

LITERATURE REVIEW and METHODOLOGY

EAHCP Project No. 146-15-HCP


Prepared by
Dr. Weston H. Nowlin
601 University Drive
Department of Biology
Aquatic Station
Texas State University
San Marcos, TX 78666
(512) 245-8794
wn11@txstate.edu

Dr. Benjamin Schwartz
601 University Drive
Edwards Aquifer Research and Data Center
Department of Biology
Aquatic Station
Texas State University
San Marcos, TX 78666
(512) 245-7608
bs37@txstate.edu

and

Parvathi Nair
601 University Drive
Department of Biology
Aquatic Station
Texas State University
San Marcos, TX 78666
p_n22@txstate.edu

INTRODUCTION AND LITERATURE REVIEW

The Edwards Aquifer Recovery and Implementation Plan (EAHCP) currently sets the long-term mean and minimum daily discharge objective for Comal Springs at 225 cfs (cubic feet/second) and 30 cfs, respectively. However, modeling results from Phase 1 of the EAHCP predict that the mean and minimum daily discharge will be 197 cfs and 27 cfs, respectively (EARIP 2012). Thus, there is currently concern about the impacts of lower spring flows on Comal Springs riffle beetle populations. Historical data and modeling results indicate some of the potential loss of habitat and habitat degradation associated with the reduction in spring flows. It has been observed that Spring Runs 1 and 2 generally cease to flow when total Comal Springs flow is ~130 cfs and Spring Run 3 generally ceases to flow when Comal Springs total flow is about 50 cfs (LBG Guyton 2004). Modeling results suggest that discharge will be less than 120 cfs for a total of 127 months and less than 45 cfs for a total of 7 months during a repeat of the drought of record (in the 1950s) with Phase 1 of the HCP implemented (EARIP 2012). Modeling efforts also indicate that a repeat of the drought of record (with Phase 1 of the HCP fully implemented) will lead to the total flows in the Comal Springs system to be < 30 cfs for a two month period (EARIP 2012). If flows drop below 30 cfs, it is expected the main spring runs in the system (Spring Runs 1 through 6) will be dry for a considerable time period and the remaining aquatic habitat within the Comal Springs system will be limited to portions of Landa Lake and the Spring Island area. Cumulatively, this information indicates that it is possible for several if not most of the spring runs in the Comal system to cease flowing for extended periods of time (from months to years) and for a significant reduction of aquatic habitat to occur if there is a recurrence of the drought of record.

The Comal Springs riffle beetle and spring flows

The Comal Springs system exhibits consistent temperatures (annual mean = 23.4 °C), high water transparency, and low nutrient and bacteria levels (USFWS 1996). Monitoring by the EAA at ~80 groundwater wells, eight surface water sites, and major springs groups across the region indicates little contamination in the aquifer. However, as total spring flow in the system declines, water quality in the remaining habitat will likely be a primary concern. Two of the most relevant water quality changes associated with reduction in flows that would potentially have an impact on the Comal Springs riffle are temperature and dissolved oxygen.

It is currently thought that the occurrence of Comal Springs riffle beetles within the Comal system is largely limited to habitats immediately adjacent to spring outflows. Therefore, a reduction in spring flow that leads to loss of habitat (via desiccation) or reduces water quality of their occupied habitat will likely impact the fitness and survival of beetles. Obviously, water quality will not be the primary issue in the Spring Runs or along the western shoreline during substantial low-flow events because these habitats will cease to flow and the habitat associated with the presence of the Comal Springs riffle beetle (i.e., areas around spring orifices) will be dry. However, in the summer period 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

surface water temperatures will increase and DO concentrations will drop. Low flow conditions in the Comal system are presumed to significantly affect the daily average water temperature and dissolved oxygen (DO) concentration. In addition, lower spring flows will likely alter the diel cycles of these and other abiotic variables in Comal Springs riffle beetle habitat.

Temperature is a critical factor affecting riffle beetles, particularly though affecting their respiration through plastron function; however, temperature strongly affects the concentration of dissolved gasses in an aqueous environment, creating interdependence between the effects of temperature and DO on plastron function and the fitness of riffle beetles. Elevated environmental temperatures increase metabolism (and thus O_2 demand) in poikilotherms, but increasing temperature also decreases gas solubility in water, resulting in lower overall DO concentrations. Both of these factors have the potential to affect plastron function because they affect passive diffusion of O_2 across the air/water interface as the pressure gradient of O_2 within the plastron is reduced during metabolic (O_2 consuming) activities. This gradient requires DO concentration in the surrounding water to be relatively high; otherwise, O_2 would diffuse out of the plastron reducing O_2 available for the organism (Brown 1987, Resh et al. 2008).

Thermal tolerances in organisms and stress responses to increasing temperatures

Temperature is one of the most important abiotic environmental factors that influences physiology, behavior and geographical distribution of animals (Fry, 1947). The environmental temperature controls body temperature (T_b), which governs vital processes in ectotherms, such as behavior, locomotion, metabolic rate, and cardiorespiratory function (Huey and Steveson, 1979; Farrell, 2002; Kiefer et.al, 1998). During acute exposure to a broad range of temperatures, an asymmetric function describes the relationship between T_b and performance, where performance is maximized at an intermediate temperature - the thermal optimum (T_o) (Angilleta et.al, 2002). Many organisms can select for a temperature that often cooincides with thermal optima for physiological processes such as growth, metabolism, locomotion, and reproduction (Beitinger and Fitzpatrick, 1979). By using thermoregulatory strategies such as sheltering and using thermal refugia, organisms can maintain body temperature at or near T_o (Thorpe, 1994).

Metabolic rate and, consequently, the oxygen consumption are directly related to the temperature in heterothermic organisms (Salvato et.al, 2001). In poikilotherms, elevation of environmental temperature increases metabolism and thus oxygen demand. Therefore, the effect of an environmental factor such as temperature can act as a stressor and the severity of the stress response by an organism can be assessed by measuring oxygen consumption (Diaz et.al, 2007). The effects of temperature on rates of respiration can be quantified by calculating temperature coefficient or Q_{10}, i.e. the effect that a 10^0C (10 K) change in temperature has on the rate in respiration. For rates of respiration, Q_{10} values near 2.0 or slightly higher (i.e., metabolic rates approximately double every 10^oC) are observed when thermal effects are studied within the species' normal range of body temperatures. However, deviations from this "rule of 2" may indicate thresholds of stress on organism. Indeed, Q_{10} values less than 1.0 at higher temperatures

may be indicative of lethal effects through damaging respiratory function and irreversible loss of function (Hochachka and Somero, 2002).

Response to temperature and dissolved oxygen in elmids

A number of past studies have examined the effects of temperature and DO on plastron function and survival of other elmid species (i.e., Harpster 1944). Recent experiments we conducted (Nowlin et al. 2014) examined responses of the *H. comalensis* and a closely-related species (*H. glabra)* and in short-term DO change experiments (minute-scale changes), both *H. comalensis* and *H. glabra* were able to tolerate low DO concentrations (~0 mg/L) for several minutes without exhibiting any negative responses. In addition, short-term temperature experiments indicated that both *H. comalensis* and *H. glabra* were able to tolerate fairly high temperatures before exhibiting behaviors that are indicative of stress [e.g., uncoordinated movement and loss of response (LOR) to an external stimulus]. However, in the short-term experiments, *H. comalensis* had significantly lower temperature thresholds than *H. glabra* for initiation of rapid movement around the experimental chamber (29^{o}C *vs*. 32^{o}C), the onset of uncoordinated movement (37^{o}C *vs*. 40^{o}C), and the onset of a loss of response (LOR; 45^{o}C *vs*. 50^{o}C). Unfortunately, both time limitations and low flow conditions in 2014 prevented us from conducting long-term DO and temperature experiments on *H. comalensis*, but long-term DO experiments indicated that onset of LOR in *H. glabra* occurred at 0.5 mg/L. Thresholds for onset of uncoordinated movement (35^{o}C) and LOR (36^{o}C) in long-term temperature experiments with *H. glabra* were substantially lower than those observed in the short-term temperature experiments, indicating that long-term exposure to temperatures >30oC likely has cumulative negative effects on riffle beetle fitness. In addition, *H. glabra* exhibited substantial mortality in the long-term experiments when temperature exceeded 30^{o}C. Overall, this previous study suggests that riffle beetles are less sensitive to changes in DO, but long-term exposure to higher temperatures are likely to lead to substantial fitness effects. However, there is still a need to perform long-term experiments on *H. comalensis* to assess its sensitivity to relatively long-term changes in environmental conditions.

More recently, in an EAHCP study conducted with BIO-WEST, we examined the effects of gradually-increasing temperatures on several species of riffle beetles, including *H. comalensis* (BIO-West 2015). Riffle beetle adults were exposed to gradually increasing temperatures over a period of several weeks (35 days). In this experiment, we observed the onset of mortality in both H. comalensis and H. glabra at 26^{o}C, whereas the generalist riffle beetle *Microcylloepus pusillus* did not exhibit onset of mortality until >29^{o}C. These results were consistent with the Nowlin et al. (2014) study, but also suggest that prolonged exposure to elevated temperatures leads to accumulated stress and mortality in adult riffle beetles and that the two spring-associated species (*H. comalensis* and *H. glabra*) exhibited significantly lower mortality thresholds to temperature than the supposed generalist species (*M. pusillus*) that has a more cosmopolitan distribution in the Comal system.

Study objectives

In this study, we will examine the individual and combined roles of relatively long-term increases temperatures and declines in DO concentrations on *H. comalensis* and several riffle beetle species native to the Edwards Plateau in an experimental laboratory-based setting. Individual adults of at least three elmid species will be collected in the wild and brought to the lab for experiments examining the effects of changing DO and temperature on beetle fitness. The main three elmid species are *Heterelmis vulnerata*, *H.* cf. *glabra*, and *H. comalensis* (Fig. 1). Although *H. comalensis* is the species of concern and a main focus of the EAHCP, we elected to additionally conduct experiments with two other closely-related species for two reasons: (1) to provide comparison of long-term temperature and DO response among elmid species that differ slightly in their habitat associations, and (2) to explore the potential to use non-listed "surrogate" species in experiments or studies that might result in the injury or mortality of individuals of the species of concern.

Potential differences among elmid species in thermal tolerances

It has been hypothesized that organisms which are found exclusively in relatively stable thermal environments should exhibit a relatively narrow range of temperature tolerances (stenothermal). Conversely, in a more thermally fluctuating environment, organisms should evolve to a eurythermal profile, with their thermal optima extended throughout a broader range of temperatures (Issartel et.al, 2005; Huey and Kingsolver, 1989). The three species examined by this study are likely to exhibit differences in thermal optima. *Heterelmis vulnerata* is found throughout the Guadalupe River basin and is morphologically similar to *H. comalensis*, but differs slightly in its ecology by inhabiting surface water dominated streams (R. Gibson, *pers. obs.*). Thus, *H. vulnerata* potentially provides a comparative species that is adapted for more variable DO and temperature conditions than spring-associated species (e.g., *H. comalensis*).

Heterelmis cf. *glabra* is the most closely-related species to *H. comalensis* and shares both morphological and ecological similarities (i.e., spring outflow association). Populations of *H.* cf. *glabra* reside around spring outflows of the Devils River (R. Gibson, *pers. obs.*) and are presumably spring-adapted like *H. comalensis*. Therefore, *H.* cf. *glabra* has the potential to serve as a "surrogate" species for *H. comalensis* in potentially harmful experiments, but the potential for this remains largely unknown.

Microcylloepus pusillus is another elmid species that occurs in the same habitat as *H. comalensis* in the Comal system, but it not as strongly associated with the presence of spring openings. At this point, it will not be one of the focus species in this study because previous work indicates that it has substantially larger thermal tolerances than *H. comalensis* (Bio-West, 2015). In addition, recent stable isotope studies indicate that *Microcylloepus pusillus* is likely not the best candidate to serve as a surrogate species for the Comal Springs riffle beetle because it utilizes different food resources (Bio-West, 2015).

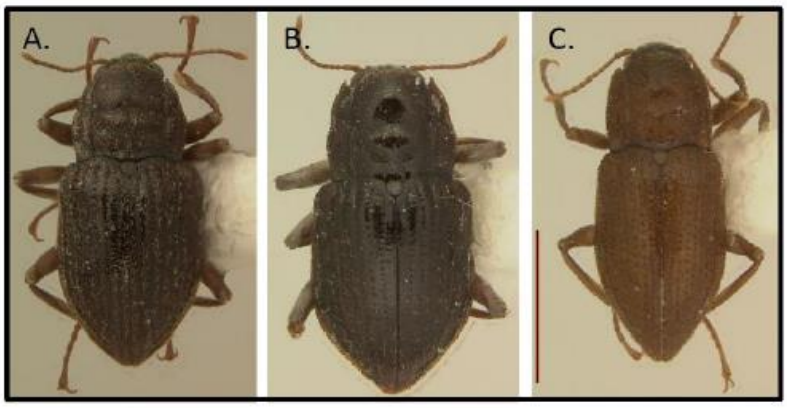

Fig. 1 The three riffle beetle species collected for use in this study. (A.) *H. glabra*, (B.) *H. vulnerata*, and (C.) *H. comalensis*.

METHODS

Collection and housing of beetles

Experiments examining riffle beetle responses to changes in DO concentration and temperature will be performed in temperature-controlled living stream systems at the Freeman Aquatic Biology Building (FAB) at Texas State University. Adult and larval beetles of each species will be collected in the wild (by hand picking or with cloth lures), transported to the lab, and maintained in temperature controlled recirculation systems with a constant addition of Edwards Aquifer well water for at least 2-weeks before being used in experiments. For all species, collected individuals will be placed into PVC tube containers and the containers will be placed into coolers filled with water from the source location and transported to the lab. In the labs, populations of adults will be housed in flow through chambers held within living stream systems and acclimated to temperatures set at approximately spring outflow temperatures (~23^{o}C) prior to the start of experiments. Larvae will be housed separately from adults, but the same handling and housing procedures will be applied to larvae. Riffle beetle populations will be kept in plastic flow through chambers which contained pre-cleaned limestone river cobbles, well-conditioned terrestrial detritus (leaves and twigs; their presumed food source), and cotton-poly rags.

Recently, there has been a concern about the state of the water quality in FAB; due to unknown reasons, adult riffle beetles have experienced substantial mortality over the last year, but larvae are not affected. Currently, we know that the downstairs Holding House water does not cause mortality in adult beetles and that the Wet Lab water does not cause mortality for adults when it is first passed through a flow-through activated charcoal filter. We have received assurances from Texas State University Facilities that they will work with us to determine the source of the problem, fix the problem, and that they will supply us with whatever charcoal filtration needs we require so that we can successfully conduct any future riffle beetle research as they work on the issue. Thus, as a

part of this proposed research, any experiments conducted in the wet lab will first pass all water through charcoal filters before it comes into contact with beetles.

Conceptual foundation for experiments and experimental designs

For this study, we will conduct two main experiments which assessed the effects of temperature and DO on riffle beetles. The first set of experiments will assess the separate effects of slowly increasing temperature and declining DO concentrations on riffle beetles using Critical Thermal Methodology (CTM) (*sensu* Beitinger et al. 2000). Critical Thermal Methodology is a common experimental approach to assessing organismal environmental tolerances. In CTM studies, an individual organism is exposed to a linear increase or decrease in temperature until a defined sub-lethal endpoint is reached. The endpoint is the temperature (or DO concentration) at which an observable response, such as lack of movement or loss of muscular control, is reached (Fig. 2).

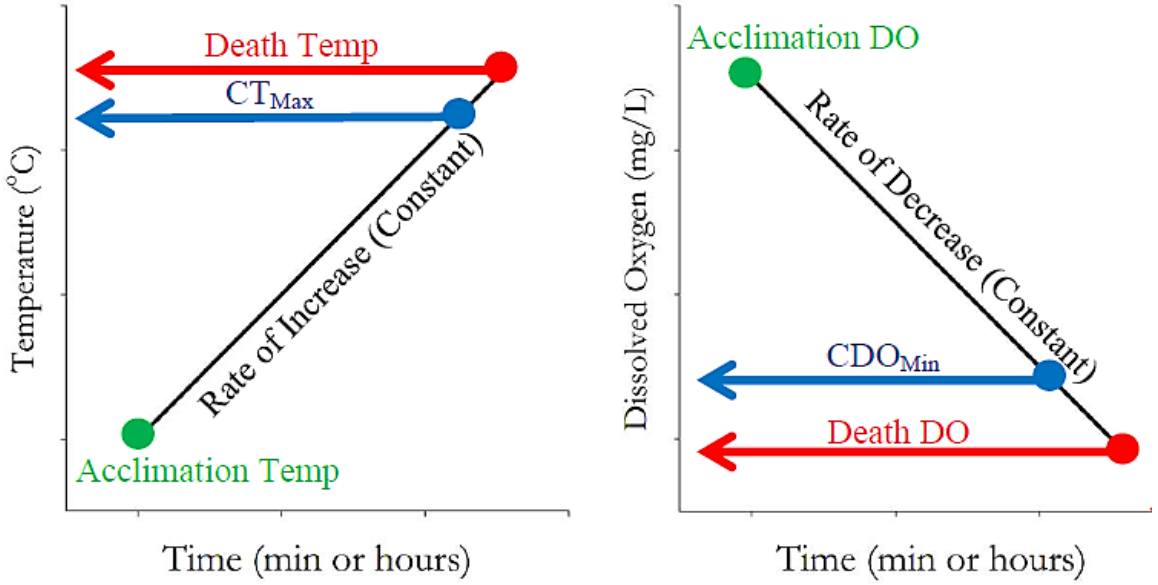

Fig. 2 Conceptual diagram of determination of critical maximum for temperature (CT_{Max}) and minimum for DO (CDO_{Min}). Temperature or DO is changed at a constant (linear) rate over a period of time until the critical endpoint is reached. The critical endpoint is a clear response that occurs prior to physiological death. Once the endpoint is reached, the organism is removed from experimental conditions and placed back into the acclimation conditions and monitored for recovery.

The first set of experiments will assess riffle beetle responses to relatively slower changes in temperature and DO (over a daily basis). By gradually changing temperature or DO concentration over 24-h, individual animals are provided the opportunity to acclimate to conditions prior to the next change, thus this experiment may gain a better estimate of the overall limits to the acclimation ability of an organism and generate a better estimate of the ultimate temperature and DO tolerances

of the organism in question (Beitinger *et al*. 2000). The longer experimental duration and slower rate of change may also allow time for the more cumulative and chronic deleterious effects to manifest themselves in experimental animals. It is critical to note that this set of experiments on *H. comalensis* will be conducted in exactly the same manner as the long-term experiments that were conducted *on H. glabra* in the previous study we performed for the EAHCP. All methods, equipment, and procedures will be exactly the same so that the results generated here can be directly compared to and integrated with the results of the previous study**.**

For this set of experiments, beetles will be first acclimated to 23ºC (ambient spring water temperatures) and >4 mg DO/L (~4 mg DO/L is ambient DO of emerging spring water) prior to the start of experiments. Decreases in DO concentrations will be accomplished through the introduction of bubbled N_2 gas to drive off DO and lower DO concentrations to desired levels (Martinez *et al.* 1998, Ostrand and Wilde 2001, Chiba *et al.* 2004, Denisse and Diaz 2011). In order to maintain or alter the desired DO concentrations during experiments, we will utilize a Dissolved Oxygen Control System (Qubit Systems, Inc.), which has the capacity to measure DO levels in up to four channels and can control the DO level automatically through the release of N_2 or O_2 (or standard laboratory air). Changes in temperature will be performed by temperature control units attached to the living streams (Frigid Units, Inc.).

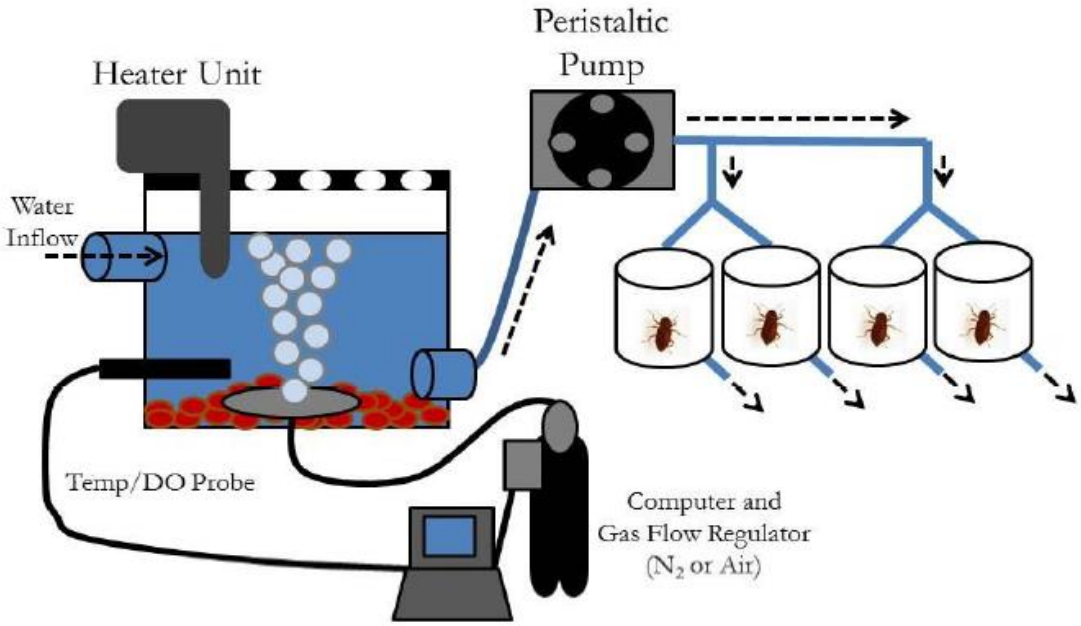

Fig.3 Schematic of the system to be used for Critical threshold experiments. The DO Control System allows for continuous and automatic feedback of the DO concentration in the initial water reservoir and will trigger the bubbling of N_2 or Standard Air, depending on the desired and pre-set DO concentration. Incoming water temperature is ~23ºC, and changes to water temperature will be accomplished by setting the desired temperature on the Heater Unit.

Long Term Experiments: Determination of Critical Thresholds to Rapid Water Quality Changes

For the first set of experiments, the overall set up will be the same as the previous work we conducted (Figure 3). Edwards Aquifer water will be continuously supplied to a 5-L plastic tub immersed in a living stream. The plastic tub has a layer of aquarium gravel in the bottom and a large aerator disc. The temperature control unit will be placed in the initial reservoir and will be set to the desired temperature. The temperature and optical DO probe for the DO Control System will be placed in the reservoir and continuously recorded DO and temperature and provide feedback to the system so that it automatically regulates DO concentration. The DO/temperature probe is connected to a laptop which interfaces with an automatic gas flow regulator that regulates the flow of N_2 or standard air into the reservoir chamber, depending on the desired DO concentration. The top of the reservoir is perforated to allow degassing. An outflow port located below the water line is attached to Tygon tubing which leads to an adjustable peristaltic pump, which draws water from the reservoir and then leads to two 2-way splitters in the line – each line then leads to an airtight high density polyethylene (HDPE) chamber that has inflow and outflow lines and houses individual beetles during experiments. The openings to the inflow and outflow tubes to the chambers are covered with a fine mesh to prevent escape. The entire system is closed to the atmosphere from the outflow of the initial reservoir to the point of discharge from the beetle holding chambers, thus the DO concentration in the beetle chambers is the same as the concentration measured in the initial reservoir. For all experiments, the flow rate through chambers will be ~60 mL per minute to allow complete replacement of the chamber volume every 1 minute. In accordance with most CTM studies, the presumed sub-lethal but clearly identifiable endpoint for experiments is a Loss of Response (LOR) to a stimulus. Thus, for each individual beetle, we will record the endpoint temperature or DO concentration at which an LOR is observed. Because chambers are largely closed to the atmosphere, the stimulus will be the gentle agitation of the chamber; initial observations indicate that gentle agitation will cause beetles to move (i.e., a response). We will additionally measure the respiration rate of adult beetles (and potentially larvae) at each temperature step using a Qubit system interfaced with a respiratory chamber (Fig. 4). In order to avoid pseudoreplication issues, once we use an individual beetle in an experiment, it will not be used in any subsequent experiments.

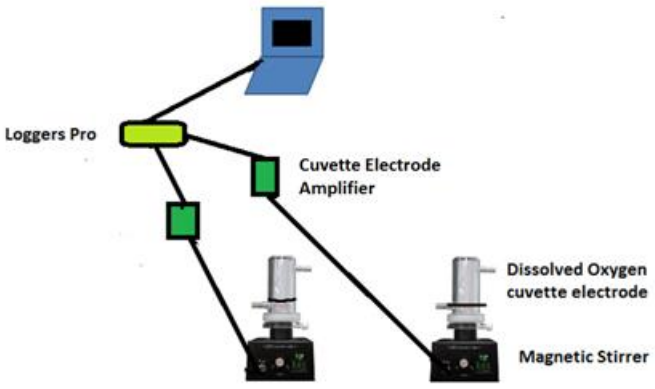

Fig. 4 Schematic of the system to be used for measuring respiration rates

Experiments assessing the effect of unchanging environmental conditions on beetle fitness

In the second set of experiments, we will assess adult and larval riffle beetle responses to long-term and persistent conditions in order to assess how fitness is affected by continuous and persistent high temperature or low DO environments. Thus, this set of experiments does not attempt to determine Critical Thresholds, but rather examines how beetle survival and stress is influenced by persistent and unchanging environmental conditions over relatively long periods (≥2 months). The experimental design will examine a range of temperatures and DO concentrations on beetle fitness that are representative of the hypothesized and observed conditions that exist in the Comal system during normal and low flow periods. In addition, the range in conditions is not meant to represent temperatures or DO concentrations that would be immediately lethal or cause an LOR to beetles that we determined in the previous short-term experiments. For temperature experiments, we will house groups of individual beetles (adults and larvae) at the following temperatures: 23°C, 26°C, 29°C, and 32°C. For DO treatments, we will house beetles at the following concentrations: 4 mg/L, 3mg/L, 2 mg/L, and 1 mg/L.

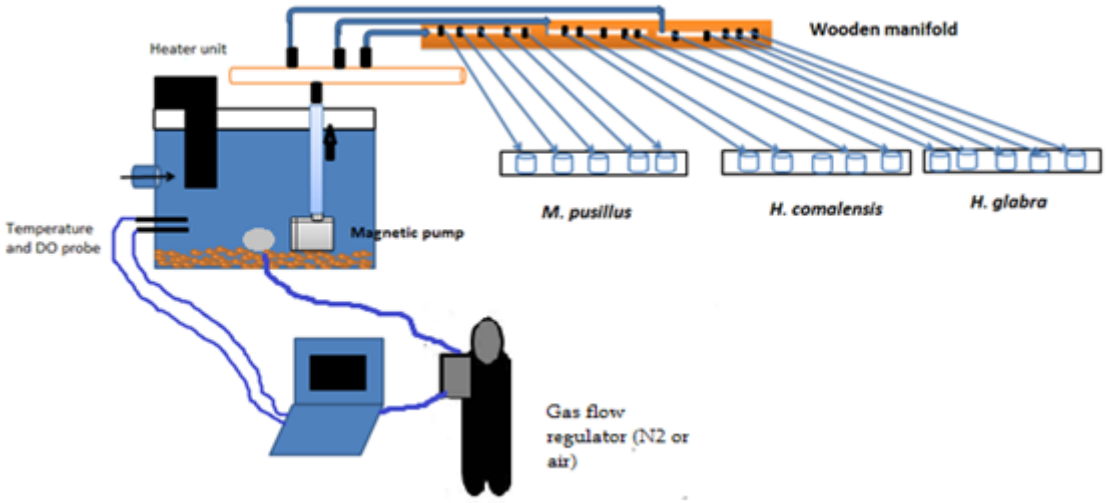

Fig. 5 Schematic of the system to be used for assessing beetle

The experimental set up will include a 5-L plastic tub which lay within a living stream with continuous supply of Edwards Aquifer water (Fig. 5). The plastic tub will have a layer of pea-sized aquarium gravel in the bottom, an aerator disc, and a magnetic drive pump (~700 gph or 44 liters per minute; Pentair Aquatic ecosystem, Model MD7). A temperature control unit (Frigid Units, Inc.) will be placed in an initial reservoir and could be set to the desired temperature via digital interface. The temperature and optical DO probe for the DO Control System will be placed in the reservoir and continuously recorded DO and temperature and provide feedback to the system so that it automatically regulates DO concentration. The DO/temperature probe is connected to a laptop which interfaces with an automatic gas flow regulator that regulates the flow of N_2 or

standard air into the reservoir chamber, depending on the desired DO concentration. The top of the reservoir is perforated to allow degassing. The outlet of the magnetic pump will be connected to flexible clear vinyl tubing which will lead to a manifold with multiple splitters in the line – each line led to an airtight high density polyethylene (HDPE) chamber that had inflow and outflow lines and would serve to house beetles during experiments. Openings to the inflow and outflow tubes to the chambers will be covered with a fine mesh (100μm aperture) to prevent beetle escape. Individual beetles of each species will be housed in individual HDPE chambers that were suspended in a living stream using a plastic screen mesh. Three plastic screens were suspended in the living stream and each plastic screen with 5 cups housed 5 individuals of the same species (one individual in each cup). Replacement of water into each beetle holding chamber was estimated at 120 mL per minute, allowing for complete replacement of the chamber volume every 30 seconds.

Each temperature or DO treatment will contain 5 replicate chambers. Once adult and larval beetles are brought into the lab and after a 2-3 week equilibration period at ~23^{o}C, they will be placed into the group chambers and slowly acclimated to the temperature or DO concentration of the treatment (change temperature or DO by 1^{o} or 1 mg/L per day). Temperature and DO targets will be met with the use of an automated Qubit system (for DO) and a temperature control unit. Once the target temperature or DO is reached, beetles will be maintained under those conditions for at least a two-month period. Each replicate group chamber will contain a standard amount of leaf litter (food source) and cotton-poly cloth. After reaching target conditions, chambers will be checked approximately every 2-3 days to assess the number live and dead individuals (estimate % survival) and any dead individuals will be removed. We will also periodically remove individuals from the temperature treatments and assess their metabolic activity and stress through measuring their respiration rate with a Qubit system and a small volume respiration chamber (see description of set up above).

Data Analysis

For the first set of experiment, the critical temperature and DO thresholds of *H. comalensis* and of any other species examined will be determined as the arithmetic mean of the LOR endpoints of each individual beetle used of each species. Differences among species or life stages (adult versus larvae) for DO and temperature endpoints will be assessed with one-way ANOVA. Data will be examined to determine if they met assumptions of normality and heterogeneity of variances. In addition, critical temperature and DO thresholds will be compared to data in the literature for other elmid and dryopid beetle species (Harpster 1941, 1944).

For the second set of experiments, we will compare the % survival of beetles across the various temperatures and DO concentrations using one-way ANOVA. Data will be examined to determine if they met assumptions of normality and heterogeneity of variances. In addition, survival of *H. comalensis* and any other species will be compared to data in the literature for other elmid and dryopid beetles (Harpster 1941, 1944).

Most importantly, we will conduct a review of 14 years of existing Comal springs temperature and DO data from the Biological Monitoring program. We will establish the natural and observed range in temperatures and DO in the system during different flow conditions and then directly compare those conditions to the Critical Thresholds we observed in experiments and the temperature- and DO-survival data we generate in the second set of experiments.

LITERATURE CITED

Angilletta, M.J., Niewiarowski, P.H., Navas, C.A. 2002. The evolution of thermal physiology in ectotherms. J. Therm. Biol. 27, 249 – 268Brown, H. P. 1987. Biology of riffle beetles. *Annual Review of Entomology* 32:253-73.

Beitinger, Thomas L., and Fitzpatrick, L.C. 1979. Physiological and Ecological Correlates of Preferred Temperature in Fish. *American Zoologist* 19 (1). Oxford University Press: 319–29.

Beitinger, T. L., W. A. Bennett, and R. W. McCauley. 2000. Temperature tolerances of North American freshwater fishes exposed to dynamic changes in temperature. Environmental Biology of Fishes 58: 237–275.

Biowest, Inc. 2015. Comal springs riffle beetle habitat connectivity study – Final Report. Edwards Aquifer Authority HCP Study no. 132-14-HCP.

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

Brown, H. P. 1987. Biology of riffle beetles. Annual Review of Entomology 32:253-73.

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

Chiba, S., L. Aoki, and T. Ogata. 2004. Response of the pandalid shrimp *Pandalus latirostris* to dissolved oxygen, salinity and turbidity. Fisheries Science 70:1174-1176

Díaz, F., Denisse, Re A., González, R.A., Sánchez, L.N., Leyva, G., Valenzuela, F. 2007. Temperature preference and oxygen consumption of the largemouth bass *Micropterus* salmoides (*Lacépéde*) acclimated to different temperatures. Aquac. Res. 38, 1387–1394.

Denisse, A., and F. Diaz. 2011. Effect of different oxygen concentrations on physiological energetics of blue shrimp, *Litopenaeus stylirostris* (Stimpson). *The Open Zoology Journal* 4:1-8.

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

Farrell, A.P. 2002. Cardiorespiratory performance in salmonids during exercise at high temperature: insights into cardiovascular design limitations in fishes. Comparative Biochemistry and Physiology – Part A: Molecular and Integrative Physiology. 132, 797–810

Fry, F. E. J. 1947. Effects ofthe environment on animal activity. Univ. Toronto Studies Biol. Series, No. 55; Pub. Ont. Fish. Res. Lab., No. 68:1-62.

Harpster, H. 1941. An investigation of the gaseous plastron as a respiratory mechanism in *Helichus striatus* Leconte (Dryopidae). *Transactions of the American Microscopical Society* 60(3): 329-358.

Harpster, H. 1944. The gaseous plastron as a respiratory mechanism in *Stenelmis quadrimaculata* Horn Dryopidae. *Transactions of the American Microscopical Society* 63(1): 1-26.

Hochachka, P.W., Somero gn. 2002. Biochemical adaptation: mechanism and process in physiological evolution.84.

Huey, R.B., Kingsolver, J.G., 1989. Evolution of ectotherm performance. Trends Ecol. Evol. 4, 131 – 135.

Huey, R. B., and Stevenson, R. D. 1979. Integrating thermal physiology and ecology of ectotherms. Amer. Zool. 19:357-366.

Issartel, J., Hervant, F., Voituron, Y., Renault, D. and Vernon, P. 2005. Behavioural, ventilatory and respiratory responses of epigean and hypogean crustaceans to different temperatures. Comp. Biochem. Physiol. A. 141, 1-7.

Kieffer, J.D., Alsop, D. & Wood, C.M. 1998. A respirometric analysis of fuel use during aerobic swimming at different temperatures in rainbow trout (Oncorhynchus mykiss). Journal of Experimental Biology. 201, 3123–3133

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

Martinez, E., M. Aguilar, L. Trejo, I. Harnandez, E. Diaz-Iglesia, L. A. Soto, A. Sanchez, and C. Rosas. 1998. Lethal low dissolved oxygen concentrations for postlarvae and early juvenile

Penaeussetiferus at different salinities and pH. *Journal of World Aquaculture Society* 29(2): 221-229.

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

Ostrand, K. G., and G. R. Wilde. 2001. Temperature, dissolved oxygen, and salinity tolerances of five prairie stream fished and their roles in explaining fish assemblage patters. *Transactions of the American Fisheries Society* 130:742-749.

Resh V. H., D. B. Buchwalter, G. A. Lamberti, and C. H. Eriksen. 2008. Aquatic insect respiration. Pages 39-53 *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.

Salvato, B., Cuomo, V., Muro, P.Di., Beltramini, M. 2001. Effects of environmental parameters on the oxygen consumption of four marine invertebrates: a comparative factorial study. Mar. Biol. 138, 659 – 668.

Thrope, J. E. 1994. Salmonid flexibility: responses to environmental extremes. Transaction of the American Fisheries Society, 123,606-612.

United States Fish and Wildlife Service (USFWS). 1997. Endangered and threatened wildlife and plants; final rule to list three aquatic invertebrates in Comal and Hays counties, TX, as endangered. Federal Register 62 (18 December 1997): 66295-66304.

United States Fish and Wildlife Service (USFWS). 2007. Endangered and threatened wildlife andplants; 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.

Salvato, B., Cuomo, V., Muro, P.Di., Beltramini, M. 2001. Effects of environmental parameters on the oxygen consumption of four marine invertebrates: a comparative factorial study. Mar. Biol. 138, 659 – 668.