Final Project Report

Conservation Physiology of *Chaetodipus penicillatus sobrinus*

Prepared For:

Desert Conservation Program
Clark County Department of Environment and Sustainability
Caryn Wright, Environmental Specialist
4701 West Russell Road, Suite 200
Las Vegas, Nevada 89118

Prepared By:

Sean A. Neiswenter

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EXECUTIVE SUMMARY

Project Overview: This study evaluated the conservation physiology of the desert pocket mouse (*Chaetodipus penicillatus sobrinus*) in southern Nevada. As a peripheral isolate, populations of *C. p. sobrinus* potentially face significant threats from the escalating risks of climate change. This project utilized respirometry, transcriptomics, and behavioral observations to assess how increasing temperatures and variable precipitation impact the species' physiological limits and adaptive capacity.

Key Research Findings:

Metabolic and Physiological Response (Respirometry)

- **Basal Metabolic Rate (BMR):** The species maintains a stable BMR between 29°C and 37°C, identifying its thermal neutral zone. However, metabolic rates significantly increase at lower temperatures (23°C) and during periods of extreme heat stress.

- **Heat Stress & Hyperthermia:** When exposed to 37°C (simulating nocturnal or burrow temperatures during a summer heatwave), individuals experienced a peak in body temperature (heterothermy) up to 41°C. This physiological strain resulted in a metabolic rate 2.25 times higher than baseline, indicating a high energetic cost to surviving extreme heat.

- **Water Loss:** Low humidity environments (simulating drought) caused a **nine-fold increase** in water loss compared to control conditions. Interestingly, water loss remained constant across the tested temperature range, suggesting that humidity, rather than temperature alone, is the primary driver of dehydration risk.

Genetic Indicators of Stress (Transcriptomics)

- **Tissue-Specific Sensitivity:** Analysis of brain, kidney, and skeletal muscle tissues revealed significant changes in gene expression under heat and water stress.

- **Brain Vulnerability:** The brain showed the lowest transcriptomic response, indicating it is the organ most sensitive to environmental fluctuations. These changes suggest potential subclinical impacts on cognitive functions such as decision-making and motor control.

- **Adaptive Signaling:** Dehydration triggered important gene expression changes in the kidneys to manage water balance.

- **Climate Risks:** Climate forecasts predict a **5°C increase** in soil temperatures at 50cm depth over the next 50 years. Behavioral experiments were conducted to determine if the species possesses the plasticity to dig deeper burrows to escape rising subsurface heat or if they are genetically constrained to specific burrow depths.

Conclusions and Conservation Risks

The study provides evidence that drought is likely to be more of a threat than temperature over the next 50 years. The main threat identified in this study is low humidity during foraging. Individuals may manage the threat in a variety of behavioral ways. Based on future climate predictions and current field measurements, *C. p. sobrinus* will likely be able to burrow to ideal conditions for daily refuge.

1 INTRODUCTION

1.1 Project Background and Need

The Clark County Desert Conservation Program (DCP) is currently pursuing a major amendment to the Clark County Multiple Species Habitat Conservation Plan (MSHCP) and associated Section 10(a)(1)(B) incidental take permit. The COUNTY has several objectives for the MSHCP Amendment, but the two that are most pertinent for this scope of work are:

- To revise the list of species covered by the MSHCP and associated incidental take permit in order to focus mitigation efforts on those species most impacted by private-land development activities; and
- To revise the conservation strategy to address the conservation needs of new Covered Species and to improve mitigation effectiveness and program accountability.

The desert pocket mouse (*Chaetodipus* penicillatus) has been identified as a species of conservation concern that warrants status as a Covered Species under the MSHCP Amendment. Over the last 3-4 decades the Las Vegas Valley has experienced expansive development leading to considerable fragmentation and habitat loss for this species. Previous genomic and distributional studies conducted for the County on the desert pocket mouse have shown that in Clark County there are 2 geographically isolated populations of the subspecies *C. p. sobrinus* with relatively small effective population sizes (BEC 2023). The continued development of southern Nevada in combination with predicted climate change leads to uncertainty in the outcome of conservation efforts for this species. However, there are unanswered questions about the potential risks imposed by climate change. This study is intended to increase our understanding of the long-term viability of populations of *C. p. sobrinus* in southern Nevada.

1.2 Project Goals and Objectives

Our goal is to determine the impact of hotter temperatures and more variable precipitation on *C. p. sobrinus* to better understand how this species will tolerate extreme weather conditions in the Las Vegas Valley. This data will be used as part of a risk assessment in a conservation strategy for the species in Clark County, Nevada.

The specific objectives of this project are:

1. Document the effects of temperature and water stress on metabolic rate and water loss in *C. p. sobrinus*
2. Identify the subclinical consequences of short-term climatic extremes in *C. p. sobrinus*
3. Determine the potential risk future climate predictions pose to long-term viability of the *C.p. sobrinus*

2 METHODS AND MATERIALS

2.1 Specimen Collection and Processing

We live trapped 25 individuals from along the Muddy and Virgin Rivers for use in this study. To determine if seasonal acclimation exists, we divided our sampling in to a “winter acclimated” cohort (Jan-April 2025 collection) and a “summer acclimated” cohort (June-July 2025 collection). Sherman live traps baited with a mix of peanut butter and rolled oats were set an hour prior to sunset in appropriate habitat.

In colder months, traps were checked every 1-2 hours throughout the night until temperatures dropped below 5°C when trapping would stop. In warmer months, traps would be checked within an hour of sunrise. Any *C. p. sobrinus* were transported back to the animal care facility at the University of Nevada, Las Vegas. Upon arrival animals were weighed, sexed, and given a unique ID. Winter acclimated animals were initially housed at room temperature (approximately 23°C). The use of torpor at room temperature interfered with respirometry experiments so animals were moved to a warmer enclosure at 28°C. Animals were housed individually on dried sand and offered dried millet, rolled oats, lettuce and water ad libitum. Animals were then quasi-randomly assigned to 1 of 4 experimental groups for transcriptomics ensuring at least 2 of each sex were in each group (see below experimental design). All animal handling was conducted under a Nevada State Permit issued to S. A. Neiswenter and UNLV IACUC Protocol#01233

2.2 Respirometry

2.2.1 Experimental Design

To monitor body temperature, we either implanted animals with a subdermal temperature sensitive PIT (passive integrated transponder) tag or a radio frequency reporting tag (1.5g DST nanoRFT, StarOddi, Garðabær, Iceland) implanted in the peritoneal cavity. Both methods provided similar body temperature measures; however, they each have their own drawbacks. The PIT tags are inexpensive and can be easily implanted without surgery. There are several downsides to using the PIT tags. Measurements are not automated so each measurement had to be taken by hand and transcribed along with other notes. It can be difficult to get readings when the animal is in certain positions which often would require close proximity and disruptive handling to get a measurement. We also had problems with consistency and accuracy of readings with some tags. During respirometry experiments it is necessary to not disturbed the animal or risk impacting metabolic rate measurements. This largely negated using the PIT tags to monitor body temperature real time during respirometry but PIT tagged animals were still monitored during husbandry and before and after each experiment. To obtain real-time measurements of body temperature remotely we recorded data being reported from the DST nano tags at 1 min. intervals using program Mercury (StarOddi, Garðabær, Iceland). These tags do not have the draw backs that the PIT tags have although they do come with other issues. The DST nano tags are active and therefore require power to automatically record and transmit body temperature data which makes them significantly larger and more expensive. To accommodate the larger size, tags must be implanted into the peritoneal cavity which requires surgery and postoperative care and healing lengthening the amount of time animals are housed before experiments can commence. We used a combined approach to minimize the drawbacks of these two methods. To balance the expense and increase in time associated with the DST nano tags we only obtain real-time body temperature measurements strategically on a subset of 5 animals during higher temperature experiments coupled with animals that were only PIT tagged.

To determine the effect changing climate might have on energetics of *C. p. sobrinus* we estimated metabolic rate and water loss of individuals across a range of environmental conditions using respirometry. We used a Field Metabolic System (FMS, Sable Systems International) to measure O2 and CO2 as proxies for metabolic rate and we measured water vapor pressure to determine water loss. Animals were closely monitored for any signs of stress, such as not eating or abnormal behavior, for at least 3 days following capture before conducting any respirometry experiments. Prior to each experiment animals were placed within the respirometry chamber and allowed to acclimate in the dark for at least an hour until they appeared relaxed and settled. A wire mesh floor was placed in the cage above a layer of mineral oil to trap any urine and feces to limit the impact of these on the water vapor pressure measurements. Animals were rotated through randomized temperature and humidity experiments with at least one full day between different experiments. We were particularly interested in environmentally relevant temperatures around the thermal neutral zone and higher. Based on previous respirometry of *Chaetodipus* species we decided to focus respirometry on 8 temperature treatments at 2°C increments

from 29℃ -37℃. This range includes the typical nightly temperatures reported by official local weather stations during the summer in Mesquite and Las Vegas, NV. Previous research reported mortality of *Chaetodipus* species above 40℃ and in our trial experiments animals began showing signs of distress above 37℃. The purpose of this project was not to determine the lethal temperature or to test the limits of *C. p. sobrinus* but to determine how individuals will respond to elevated climate conditions that they will likely experience so we limited our upper temperature experiment to 37℃. Due to time constraints, 33℃ was not measured for summer acclimated animals because it was in the middle of the thermal neutral zone and we wanted to focus on the upper limit. We also included 2℃ as a lower end temperature outside of the thermal neutral zone that an animal would likely experience in a cool burrow. Humidity treatments included ambient humidity as our Control, which typically measured 22% relative humidity (RH) in the lab (range 17-30RH) and a Low humidity which involved drawing air first through a column of Drierite to lower humidity to about 5% RH before entering the respirometry chamber. Based on weather station data our Control humidity is likely the lower end of nightly conditions in the field and probably represents the extreme an animal would currently experience around dusk. We felt this was an appropriate condition for estimating future conditions where precipitation becomes more variable. Our low humidity treatment might represent the most extreme drought conditions. The experiment began with a 3-minute baseline period followed by 12-minute sampling of the animal chamber and then a final 3 -minute baseline. Data was collected at 1 second intervals using ExpeData (Sable Systems International).

2.2.2 Data Analysis

Measurements from the FMS were transformed prior to analysis. All transformations were conducted in ExpeData (Sable Systems International) using macro scripts developed at UNLV. We transformed CO2 percentage into VCO2 with the following equation: $VCO2= FR_e[(F'_eCO2– F_iCO2) + F_iCO2(F_iO2– F'_eO2)]/(1+ F_iCO2)$. VCO2 data was taken from the lowest most stable two-minute period during each experiment. For simplicity we only report VCO2 data as a proxy for metabolism although O2 data provided comparable results. The difference in water vapor pressure (dWVP) was calculated by subtracting the average of WVP during the same low stable point used for VCO2 from the baseline average WVP. We visualized data using graphs created in Excel.

2.3 Transcriptomics

2.3.1 Experimental Design

To determine the subclinical effect of short-term heat and water stress in *C. p. sobrinus* we examined the transcriptomics of different tissues from animals under temperature and water stress. Animals were randomly assigned to either Control or 1 of 3 experimental groups. Our experimental groups were intended to simulate realistic short-term climatic extremes that an animal might experience in the Mojave Desert. The temperature treatment involved exposure to 37 ℃ for up to 4 hours, simulating the early part of a summer night when an animal would be foraging during a heat wave. During initial temperature treatment trials, it was determined that animals first experienced a peak in heterothermy (body temperature elevated up to 41 ℃) during the first 2 hours of exposure at 37 ℃ after which body temperature declined back to normothermic over the latter 2 hours. To investigate this physiological response, we euthanized 2 (of the 5) individuals at the 2hr point during peak heterothermy during both the temperature and temperature x dehydration treatments and the other 3 in each group were euthanized at the end of the 4 hr. temperature treatment. The dehydration treatment involved exposure to low humidity air (~5-10% Relative Humidity) for up to 12 hrs. representing the extreme relative humidity that might be experienced during an extreme ecological drought. The final treatment was temperature x dehydration

which involved animals first exposed to dehydration then to the temperature treatment. Animal body temperature and physiological state were closely monitored during all treatments.

Following treatment, animals were euthanized and tissues were collected for transcriptomic analysis. Euthanasia was through overdose of isoflurane followed by bilateral pneumothorax. Tissues were collected systematically in the same order to limit variation among samples due to collection time. Tissues collected in order included: kidneys, liver, blood via cardiac puncture, heart, lungs, skeletal muscle from the rear left calf (gastrocnemius and soleus), brain, spleen, and intestine (small and large). All tissues except blood were immediately flash frozen in liquid nitrogen following collection and the entire process was completed within 7 minutes of euthanasia. Once frozen, tissues were stored in a -80 freezer for no more than a month until RNA was extracted. Blood was used to calculate hematocrit using a microhematocrit centrifuge for each individual to confirm hydration status.

We chose kidney, skeletal muscle, and brain to examine for our transcriptomics analysis. These tissues were chosen because we assumed they would be important in responding to temperature and water stress and because these tissues have been examined in other taxa. Kidney is essential to water balance so we assumed this tissue would exhibit large transcript changes during dehydration. Muscle is important for movement and fairly tolerant of elevated temperature because it generates a lot of heat during exercise. Muscle is sensitive to dehydration though so we expect to see minimal changes during heat stress but similar high response to dehydration similar to the kidney. The central nervous system is essential for performance, behavior, and maintaining homeostasis. The brain is extremely complex with vastly different functions performed in different regions. We utilized a portion of the right frontal lobe of the cerebral cortex and area that is important in planning, decision making, and motor control. We expected the greatest amount of response to both temperature and water stress in brain tissue because it is extremely sensitive to environmental changes.

RNA was extracted using Quick-RNA MicroPrep kit (ZYMO Research) with trizol reagent following the manufacturer's protocol. Samples were kept on ice during homogenization. We worked in sets of 8 to minimize RNA degradation during extraction. Extracted RNA was quantified using Nanodrop before being shipped on dry ice to Novogene Corp. for library prep and sequencing with their methodology which is briefly outlined here. Messenger RNA was purified from total RNA using poly-T oligo-attached magnetic beads. After fragmentation, the first strand cDNA was synthesized using random hexamer primers, followed by the second strand cDNA synthesis, end repair, A-tailing, adapter ligation, size selection, amplification, and purification. The library was checked with Qubit and real-time PCR for quantification and bioanalyzer for size distribution detection. After library quality control, different libraries were pooled based on the effective concentration and targeted data amount, then subjected to Illumina sequencing.

2.3.2 Data Analysis

Initial bioinformatic analysis of transcriptomic data was conducted by Novogene. We further analyzed the data using Novogene's proprietary software NovoMagic (cite). We conducted analysis of the data using established transcriptomic bioinformatic pipelines and methodology as outlined below.

Raw data (raw reads) of fastq format were first processed through fastp software. In this step, clean data was obtained by removing reads containing adapter, reads containing ploy-N and low-quality reads from raw data. At the same time, Q20, Q30 and GC content the clean data were calculated. All the downstream analyses were based on the clean data with high quality.

We chose *Perognathus longimembris* as our reference genome for mapping because there is no genome of any *Chaetodipus* currently available and *Perognathus* is phylogenetically the closest taxon available with an annotated genome. Genome and gene model annotation files were downloaded from GenBank. HISAT2 (2.2.1) was used to build the index of the reference genome, and HISAT2 was used to align paired-end clean reads to the reference genome. HISAT2 can use the gene model annotation file to create splice-aware alignments, providing better alignment accuracy compared to other non-splice alignment tools.

The mapped reads of each sample were assembled by StringTie v2.2.3 (Mihaela Pertea.et al. 2015) in a reference-based approach. StringTie uses a novel network flow algorithm as well as an optional de novo assembly step to assemble and quantitate full length transcripts representing multiple splice variants for each gene locus.

Quantification of gene expression level was conducted using featureCounts (2.0.6) to count the reads numbers mapped to each gene. Then FPKM of each gene was calculated based on the length of the gene and reads count mapped to this gene. FPKM, expected number of Fragments Per Kilobase of transcript sequence per Millions base pairs sequenced, considers the effect of sequencing depth and gene length for the reads count at the same time, and is currently the most commonly used method for estimating gene expression levels. Principal component analysis (PCA) was used to evaluate intergroup differences and intragroup sample duplication. PCA uses the linear algebra calculation method to reduce dimension and extract principal components from tens of thousands of gene variables. We performed PCA analysis on the gene expression value (FPKM) of all samples within each tissue independently.

Differential expression analysis for two conditions/groups was performed using the DESeq2 R package (1.42.0). DESeq2 provides statistical programs for determining differential expression in digital gene expression data using models based on negative binomial distribution. The resulting P-value is adjusted using the Benjamini and Hochberg's methods to control the error discovery rate. The threshold of significant differential expression : padj <= 0.05 & |log2(foldchange)| >= 1. In general, if a gene differs more than twice as much in expression in both sets of samples, we believe that such genes are differentially expressed. In order to judge whether the difference in expression between two samples is due to various errors or essential differences, we need to make a hypothesis test on the expression data of all genes in these two samples. The transcriptome analysis is performed on thousands of genes, which leads to the accumulation of false positives. The more the number of genes, the higher the cumulative degree of false positives in the hypothesis test, so the introduction of padj to the hypothesis test P-value is calibrated to control the proportion of false positives (Young et al., 2010).

We compared gene expression within each treatment group (Temperature, Dehydration, and Temperature X Dehydration) to the Control group for each tissue independently. We also compared Temperature and Dehydration groups to the combined Temperature X Dehydration group to assess combined effects. Data were visualized with volcano plots to show significant differentially expressed genes and further analyzed of using Gene Ontology (GO) and KEGG enrichment analysis. GO enrichment analysis of differentially expressed genes was implemented by the clusterProfiler (4.8.1), in which gene length bias was corrected. GO terms with corrected P-value less than 0.05 were considered significantly enriched by differential expressed genes. KEGG is a database resource for understanding high-level functions and utilities of the biological system, such as the cell, the organism and the ecosystem, from molecular-level information, especially large-scale molecular datasets generated by genome sequencing and other high-through put experimental technologies (http://www.genome.jp/kegg/). We used clusterProfiler R package to test the statistical enrichment of differential expression genes in KEGG pathways.

2.4 Behavioral and Environmental data

Although *C. penicillatus* is nocturnal thereby avoiding the extreme temperatures during the day, avoidance may not be possible under some climate predications. For example, in the Mojave Desert, climate forecasts predict a 5°C increase in soil temperatures at 50cm over the next 50 years (Bradford et al 2020). If animals are already living near their thermal maximum in their burrow, a 5°C increase in burrow temperature could be lethal. We were interested in the range of actual conditions available in sites where *C. penicillatus* occurs for comparison to the laboratory conditions in our experiments. Because animals could presumably burrow deeper to try to avoid rising temperatures in their burrow if they were not physiologically capable of tolerating the heat, we also tested how animals might respond behaviorally to climate change underground.

The data in this section was not part of the contracted project but was collected along with the activities that were conducted for this project. These data include environmental measurements from the field, as well as behavioral observations and experiments in captivity. We feel these data are useful in discussing the conservation physiology of *C. p. sobrinus* and therefore present some of the methodology in this section and use the results in our discussion of the risk of climate change to the peripheral population of *C. p. sobrinus* (and possibly the other subspecies in the Mojave Desert).

2.4.1 Environmental data

We collected environmental data about the subsurface conditions throughout the hottest (and generally drier) summer months at a study site in the Clark County Bunkerville East Parcel where there is a persistent population of *C. p. sobrinus* that has been actively monitored for several years. *Chaetodipus penicillatus sobrinus* is nocturnal which allows it to presumably avoid the extreme climate of the Mojave Desert during the day and reduce its environmental exposure to nighttime conditions, that are considerably cooler and more humid. Furthermore, *C. p. sobrinus*, being a small rodent, is capable of utilizing microhabitat conditions that exist within the broader area it occupies that will not be well represented by local weather station data. To better understand the actual range of conditions that *C. p. sobrinus* could utilize in the field we deployed temperature and relative humidity loggers at different locations within its habitat. Five EL-USB-2 temp/RH Data loggers (LASCAR electronics) were programed to record temperature and relative humidity at 1-hour intervals. We placed one logger approximately 2 cm above ground in the shade under a patch of arrowweed to measure the surface conditions a small rodent would experience while foraging aboveground. We buried the remaining 4 loggers to document potential burrow conditions at two depths (20cm and 50cm) underneath the same arrowweed patch and approximately 1 meter away in the open barren ground. We chose these depths based on previous literature on burrow depths reported for a similar species (Alvarez-Castañeda et al. 2005) to document the change in range of conditions available in the environment throughout the hottest point in the year. Loggers were deployed from May 2025 to November 2025.

2.4.2 Behavior

To assess how animals respond to increasing temperatures underground we used a novel climate-controlled burrowing chamber. The burrow chamber was modelled after the classic kid's toy ant farm and included a plexiglass front so we could observe the animal in its burrow. The back was made of thin sheet metal with copper pipe running parallel, spaced at 3 inch intervals down the entire chamber. We used hot water baths to pump water through the copper pipe to control temperature in different regions of the burrow chamber. The back was insulated with 1 inch Styrofoam insulation to limit heat loss to the air and direct heat into the chamber. The dimensions of the chamber were chosen partially due to readily available material sizes at the local hardware store but also to cover the average reported dimensions for similar species burrows and for comparison to the environmental data we collected above. The chamber was 24 inches wide by 36 inches tall (approximately 61cm X 91cm). The depth of the burrow chamber

was slightly wider than a *C. p. sobrinus* (approximately 0.5 inch, 130mm) so an animal had to construct its burrow against the plexiglass, but still wide enough animals could easily turn around. The burrow included a cage arena that was connected to the burrow chamber where animals were fed, initially introduced to the chamber, and had open space "above ground" to forage and move about. The front of the chamber was covered in a blackout curtain to maintain darkness in the burrow but allow observers visual access as necessary. The chamber was filled to about 75cm deep with sand sifted through a 60-mesh screen, approximately 0.25mm or less particle size which is the typical sized fine grain for sandy substrate species in the genus *Chaetodipus* (Hoover et al, 1977). Sand was dry sterilized in an autoclave then moistened at 250ml water per 5 lbs of dry sand. This resulted in sand that was not overly wet but could be packed by hand, using approximately 10lbs of pressure, to ensure burrows would not collapse.

The burrowing experiment was designed to determine if an animal would dig their burrow deeper if temperature in their burrow went above their thermal neutral zone. We hypothesized animals would seek comfortable temperatures (within their thermal neutral zone) when conditions in their burrow were elevated. For example, in the wild if an animal was in a shallow burrow and summer heat began heating the soil too high, we assume the animal would attempt to dig deeper to cooler temperatures. In this case, as long as the animal is not limited by the substrate it would be able to continue to avoid elevated soil temperatures associated with future climate change, albeit at an energetic cost of adding to its burrow. Alternatively, some rodents' burrow construction is known to be genetically determined (Weber et al 2013). If *C. p. sobrinus* is constrained in its burrow phenotype it may not burrow away from rising heat which could put it at risk in future conditions. We tested this hypothesis by allowing an animal to construct a burrow in the burrow chamber at neutral temperature. We monitored the length, depth, angle, and number chambers daily. Once burrow construction ceased for two consecutive days we elevated the temperature of the soil from the level of the burrow up to the surface. We then observed the animal's behavior in response.

3 RESULTS AND EVIDENCE OF THE RESULTS

3.1 Respirometry

Raw data for respirometry experiments were submitted to the County as a compressed file along with this report on 20 December 2025. We conducted a total of 83 temperature respirometry trials on 18 different animals during winter and summer. These included 59 trials on winter acclimated animals between six different animals over 3 months. With these animals we conducted 31 ambient humidity and 28 low humidity trials. We conducted 24 trials, all ambient humidity, on 12 summer acclimated animals over the course of 2 months.

We found notable differences in the water loss between low humidity and control trials, across all temperatures (Fig 1). The low humidity treatment for summer acclimated animals failed due to a leak in the respirometry equipment that made it difficult to maintain low humidity conditions consistent and therefore those data are not presented. During the winter acclimated low humidity trials *C. p. sobrinus* showed, on average, a nine-fold increase in dWVP compared to the control trials. The dWVP of *C. p. sobrinus* did not appear to increase or decrease with change in temperature in any of the trials.

We document small differences in basal metabolic rate (BMR) between summer and winter acclimated animals (Fig 2). We did not see any differences in BMR between low humidity trials and control trials in our winter cohort. As a result, both winter experimental types were combined for the purpose of comparing BMR between winter and summer acclimated animals. Our data showed BMR remaining

visibly stable as ambient temperature increased from 29℃ to 37℃. We did see a relatively higher metabolic rate in *C. p. sobrinus* at 23℃.

We did see an increase in metabolic rate alongside increases in body temperature in heat stressed *C. p. sobrinus* (Fig 3) and heat x water stress *C. p. sobrinus* (Fig 4). The heat stressed animal's body temperature reached 40.3℃ by the second hour of heat stress after which it began to decline to normothermic range. The heat x water stressed *C. penicillatus* had a lower hyperthermic peak at 38.5℃. Both animals exhibited a 1.3-fold increase in metabolic rate as body temperature elevated to 38.5℃. At 40.3℃ body temperature the metabolic rate was 2.25 times higher than BMR.

Animals regularly used heterothermy facultatively. An example of heterothermy is shown in Fig. 5. Body temperature during activity fluctuates around 36℃ with regular declines to about 28℃. The rapid increase to 40℃ at the end of the trace is associated with heat stress during the first 2 hours of the experiment.

3.2 Transcriptomics

All samples were successfully sequenced, and raw data was provided to the County on 28 September 2025 as Deliverable D08. After quality control, over 62 million reads were obtained across all samples. Data mapping rate was about 25% to our reference genome with 95.6% or reads mapping to known exons. In total, 24 different comparisons were used in each of the differentially expressed gene analyses (3 treatments vs. control + combined treatment vs. other 2 treatments + 2 different temperatures times vs control + 2 different temperature times compared to each other = 8 comparisons * 3 tissues). All of the results for each tissue and treatment combination are available in the data provided in Deliverable D08.

For clarity, we present in this report overall results of transcriptomics and highlight specific differential gene expression results for kidney comparing water stressed treatment to control. We chose this combination as a specific example of the transcriptomics results because it is perhaps the most biologically obvious combination in that the kidney is directly responsible for water balance. We also chose this combination because it is relevant to the results of our respirometry data. The dehydration treatment resulted in animals losing 3-5% body weight in water over the course of the 12 hour. treatment.

Results of overall results for differential gene expression data are presented in Figure 6. In general, brain tissue had the lowest number of differentially expressed genes across all treatments when compared to control (146-229 genes). Temperature treatment resulted in the greatest number of differentially expressed genes in the brain. In contrast, Muscle tissue differentially expressed the greatest number of differentially expressed genes (645 during water stress). Kidney had the greatest number of up regulated differentially expressed genes (368) during water stress compared with any other tissue or treatment.

The enrichment analysis results document enriched pathways characteristic of kidney function. For example, the KEGG analysis documents enriched pathways include Cortisol synthesis, Mineral absorption, Aldosterone synthesis (Fig. 7). The scatter plot of KEGG pathways in the water stressed kidney identifies Cortisol and Aldosterone pathways as being upregulated (Fig. 8) while the Mineral Absorption pathway is down regulated (not shown). The GO enrichment analysis highlights significant upregulation in pathways that include phosphorylation and enzyme activity (Fig 9). Some specific genes, of the 368 upregulated in the kidney, that are involved in stress response include Mgat3 (beta-1,4-mannosyl-glycoprotein 4-beta-N-acetylglucosaminyltransferase), Abcd2 (ATP Binding Cassette Subfamily D Member 2) and Nr4a1 (Nuclear Receptor Subfamily 4 Group A Member 1).

3.3 Environmental and Behavioral Data

3.3.1 Environmental Data

We only received data from 4 of the 5 loggers we deployed. The logger buried at 20cm in the shade of the arrowweed failed to record any data. All of the other loggers recorded data the entire time. Results from the 4 loggers are shown in Figures 10-13. Temperature and humidity at ground level are extremely variable. Temperature varied over 30°C daily up to a high of 56.5°C recorded at ground level in the shade. Relative humidity at ground level was highest just before dawn and lowest just prior to dusk, regularly getting below 20% RH in the summer. Much more stable temperature and humidity measurements were recorded below ground. With the least variability at 50cm in the shade under the arrowweed followed by 50 cm in the open barren ground, and then the most variability underground was in the open at the shallower depth of 20cm.

3.3.2 Behavior

A total of 14 animals were entered into the burrowing chamber. Seven (50%) constructed burrows the other seven remained above ground and did not participate in the experiment. Animals that did not begin burrow construction after 2 days were removed from the experiment. For the animals that did participate, burrow construction would generally commence quickly upon entering the chamber and an initial segment (130-530mm long and 75-220mm deep) of the burrow would be constructed the first night. Over the next 2-3 days animals would continue to expand their burrow and by day 4 or 5 animals would cease construction. Burrows typically descended at approximately 30 degrees and contained 1 or 2 separate chambers. When temperature was increased 6 of the 7 animals burrowed deeper. The remaining animal left its burrow and remained above ground until it was removed from the experiment. The maximum depth an animal burrowed during our experiments was 760mm just short of the bottom of the chamber.

4 DISCUSSION OF RESULTS

4.1 Respirometry

The results of the respirometry were not consistent with previous literature on the species or their close relatives (Hoover et al, 1977). Within previous literature it was shown that *Chaetodipus eremicus*, a sister species of *C. penicillatus*, cannot survive at or beyond 37°C. It appears *C. p. sobrinus* may have a slightly higher upper critical limit than *C. eremicus*. This increase in heat tolerance may be associated with adaptation to the higher temperatures in the Mojave Desert or it could be that we did not expose the animals to the heat as long as Hoover et al (1977). For our respirometry experiments we only measured gases for 15 mins. During our heat treatments for transcriptomic experiments we held animals for up to 4 hrs. We did see signs of heat stress in animals held at 37°C for extended periods of time. Winter acclimated *C. penicillatus* had relatively higher BMR when compared to summer acclimated *C. p. sobrinus* across all temperatures. The higher metabolic rate during winter is expected and is likely necessary to maintain body temperature while foraging on cold nights. Although our observations during winter trapping are that animals are rarely above ground and instead are most likely using torpor and relying on cached seeds as much as possible. In summer when ambient temperature is lower, metabolic rate is lowered to reduce heat load. The higher winter metabolic rate however could put individuals at greater risk from unusually warmer temperatures in winter months.

There was a difference in dWVP between the low humidity trials and the control trials. There was not a difference in dWVP by temperature. This would indicate that within our temperature range dWVP in *C. p. sobrinus* is more affected by humidity than by temperature. We would normally expect to see water loss increase with increases in ambient temperature. However, the lack of increase in dWVP at high temperatures may be consistent across members of the family Heteromyidae (Hinds & MacMillen, 1985). This would mean that any future increases in water stress may be more likely to be caused by decreases in humidity as opposed to increases in temperature. Within the Las Vegas valley we expect to see an increase in drought events (Bradford et al, 2020). Which may result in decreased humidity. However, *C. p. sobrinus* are known to spend most of their time within their burrows (Mantooth & Best, 2005). Their burrows are shown, from our data or others, to be much higher in relative humidity compared to air (Burda et al, 2007). This may allow *C. p. sobrinus* to avoid the worst impacts of predicted decreases in humidity.

During the temperature x dehydration experiment one *C. p. sobrinus* was apparently using torpor during the water stress portion and may have continued to do so while under heat stress. Despite this, the animal showed comparable increases in relative metabolic rate to other individuals during heat stress. A water stressed animal using torpor would not be without precedent. Other rodents have been found to use torpor to avoid periods of water stress (Zervanos & Salsbury, 2003). Additionally, the temperature during the water stress portion of the experiment was around 25°C, this is a temperature at which other members of the family Heteromyidae have been shown to use torpor regularly (Wang & Hudson 1970). It should also be noted that we show examples of *C. p. sobrinus* using torpor at temperatures as high as 28.5°C. This indicates that there is a strong possibility that *C. p. sobrinus* can use torpor in order to avoid periods of extreme water stress, even in summertime.

4.2 Transcriptomics

Our results support the hypothesis that different tissues respond to water and temperature stress differently. As expected, muscle and kidney showed considerably higher transcriptomic response to treatments than the brain. We chose a portion of the frontal lobe of the cerebrum for examination where conscious actions such as voluntary movement, planning, and other higher level brain functions occur. During heat and water stress, animals are likely to lose the ability to control these higher-level functions such as planning and coordination before basic physiological functions like water balance are compromised. Alternatively, *C. p. sobrinus* is capable of facultative torpor. If animals were attempting to utilize torpor during these stresses, we might expect to see higher level brain function reduced as a protective mechanism for this sensitive tissue. We have evidence of at least one animal (Fig. 5) using torpor during the temperature x dehydration treatment. The central nervous system is extremely complex and regulates many processes. A different region of the brain would likely result in a different outcome. For example, the hypothalamus regulates body temperature while the medulla oblongata is responsible for several vital functions like heart rate and respiratory rate. We can only speculate about the response of these regions because we did not include them however, it is probable given their vital functions that the level of response would have been similar to the kidney and muscle. Not surprisingly though each tissue has a unique response indicating the different functions performed by each of these tissues at the cellular level during stress.

We chose kidney during the dehydration treatment to highlight the cellular response to one stressor. Kidneys are essential to water balance, ion concentration, and blood pressure. During dehydration kidneys adjust ion concentration and volume of the urine and release hormones to help regulate blood pressure.

These vital functions are essential to maintaining homeostasis during dehydration stress. During dehydration blood volume declines leading to a drop in blood pressure. Among our tissues, kidney showed the largest number of upregulated differentially expressed genes during dehydration indicating a number of physiological pathways were being activated in response to the stress. One of the upregulated pathways we documented, aldosterone secretion, regulates blood pressure through reabsorption of sodium in the kidney tubules. We also documented increases in cortisol secretion pathways which is a generalized stress response that mobilizes stored resources such as glucose. The increased blood glucose levels are important for nervous system function during stresses but would also impact osmotic pressure in the blood helping to raise blood pressure. Other statistically significant pathways that were expressed during this treatment were pathways in cell signaling (e.g. MAPK) that are important in relaying essential external signals to the nucleus. A multitude of other kinase pathways and phosphorylation pathways were also upregulated. Phosphorylation and kinases are used to activate proteins to initiate metabolic processes. These pathways highlight the communication and coordination that is happening in this tissue as it responds to the dehydration stress. The upregulation of kinase and phosphorylation pathways also explains the relatively large response we see in the kidney transcriptomics compared to the other tissues. Some of the specific proteins that were activated in the kidneys are known to be important in glycosylation and protein folding (e.g. Mgat3), preventing oxidative stress (e.g. Abcd2) and regulating transcription during stress (Nr4a1). These proteins are essential to maintaining cell function during dehydration stress.

5 CONCLUSIONS

5.1 Conclusions and Risks Associated with Climate Change

Our primary goal with this project was to estimate the level of risk of future climate change may pose to the peripheral populations of *C. p. sobrinus* in the Mojave Desert. To this end, we documented the energetic cost of rising temperature and water loss associated with lowered humidity as a result of higher maximum temperatures in the summer, earlier cold season warming and associated longer periods of hot temperatures, and greater variability in precipitation. We also documented the sub-clinical cellular response to acute heat and water stress that may be representative of an extreme summer heat wave (e.g. summer 2024 had the highest temperature recorded in Clark County and longest number of days above 110^{0}F or approximately 43.3°C) which are expected to increase in frequency in the Mojave Desert. We recorded current microclimate conditions in the field that *C. p. sobrinus* would have access to understand how predicted changes will impact what is available to the species in the near future and we reported on some behavior experiments documenting how animals will respond to rising temperatures underground.

Rising temperature alone does not appear to be an important direct threat of climate change to this species. Current climate projections for the Mojave Desert indicate that soil temperatures may rise between 3 and 5 degrees over the next 50 years (Bradford et al. 2019, 2020). Current conditions above ground at night during foraging in the peak of summer heat are still well below the upper critical temperature of the species. Even if nightly temperatures were to rise an additional 5-7C ambient conditions would still be within the thermal neutral zone of *C. p. sobrinus*. Seasonal acclimation to higher temperatures would further buffer the species from high temperatures. One concern we had was that subsurface temperatures during the day could get too high. If animals were unable to avoid the extreme temperatures during the day due to biological (genetic/behavioral) or physical (e.g. substrate) constraints they could still succumb to heat-related death or metabolic issues. Our results offer a reassuring scenario that animals will continue to burrow away from rising temperatures and can burrow deep enough in the field to seek thermal refuge even with an increase in 5°C at 50cm. During short-term (1-2 hour) foraging bouts above ground during extreme heat events, an animal's muscles appear capable of managing excessive heat (transcriptomics data not presented but available in D09).

Based on our respirometry results it appears the biggest threat posed by climate change this century is reduced humidity during nightly foraging. It is predicted that we will see a decrease in both aboveground and belowground humidity (Bradford et al, 2020). *Chaetodipus penicillatus sobrinus* does not drink water and instead relies on moisture obtained from dry seeds and metabolic water. Our subsurface data and other measurements of burrow climates (Schmidt-Nielsen and Schmidt-Nielsen 1950) indicate that relative humidity in deeper burrows of *C. p. sobrinus* will remain high. In the lab, animals were housed at 22% RH for several months with no obvious problems and our respirometry data indicate water loss is still very low at that level. An animal at 50cm underground in the field would still enjoy at worst 60% RH. The high humidity in the burrow also allows dried seed caches to absorb moisture increasing water intake by the rodents.

Above ground during extreme drought conditions a foraging animal may experience some effects of water stress. At low humidity, but over a long period of time (up to 12 hours) animals can lose 3-5% of their body weight in water, which is clinically significant dehydration. Kidney function during dehydration is maintained but higher-level brain function may not be. Animals may have to restrict its activity during very low humidity to reduce water loss. This could be done by conducting multiple shortened foraging bouts or by foraging later in the night when RH increases. With higher level humidity in the burrow, animals could continue to seek refuge and moisture in cached seeds to recover lost moisture as it's unlikely they would forage for 12 hours continuously. Another mechanism they likely use to persist through extended periods of drought is the use of torpor. We documented extensive facultative torpor use in *C. p. sobrinus* on a daily basis and during heat and dehydration treatments. Future research should focus on the use of torpor as a mechanism to combat stressful environments such as water and heat stress in desert animals.

6 RECOMMENDATIONS

Our results suggest that climate change is not likely to be a significant threat to this species so research and management should focus on more immediate threats such as fragmentation and habitat quality. Based on our findings, we recommend conservation efforts that prioritize maintaining and increasing the quality of existing habitat while expanding new habitat to ensure connectivity among sites to ensure the appropriate resources are available for the species in the future. We also recommend establishing standardized long-term monitoring of populations across the landscape.

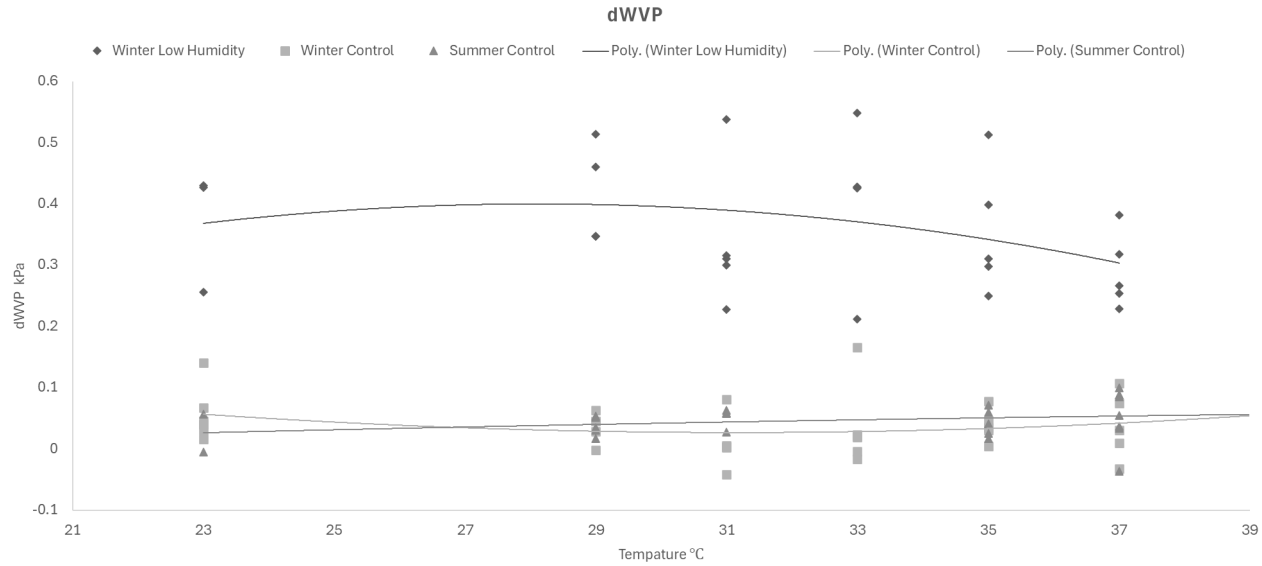

Figure 1. Evaporative water loss expressed as the difference in water vapor pressure (dWVP) between the baseline and animal chambers in control and low humidity treatments across a range of temperatures. Winter and summer acclimated groups of *Chaetodipus penicillatus sobrinus* are represented by different symbols. The summer acclimated, low humidity group is not shown due to errors during data collection (see text for more details).

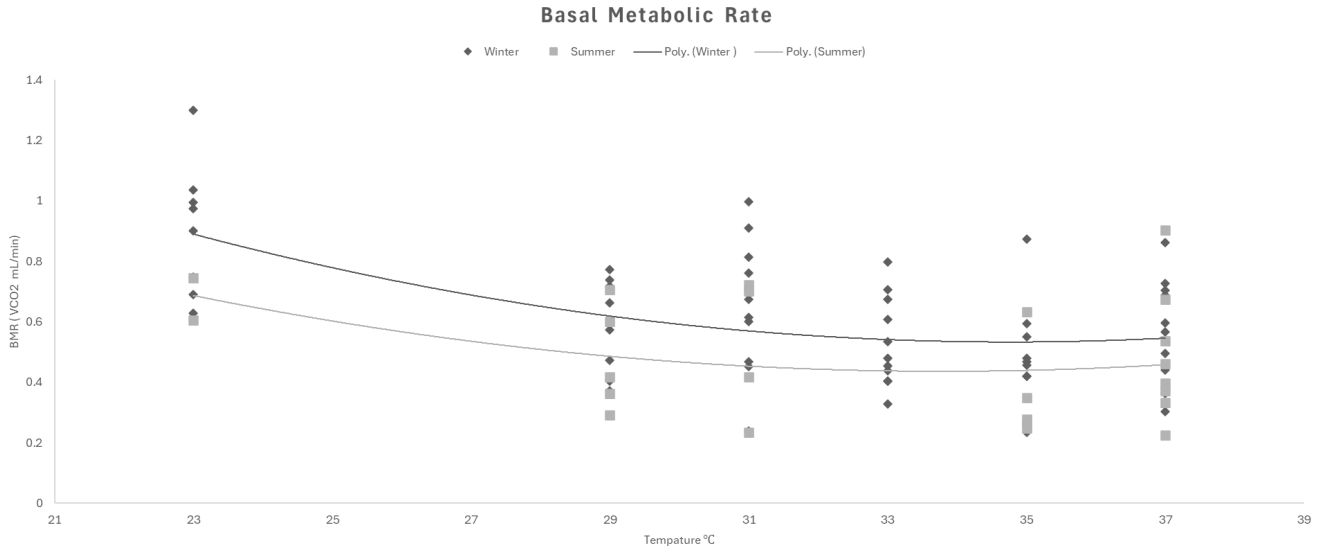

Figure 2. Basal metabolic rate (BMR) expressed as VCO2 in mL/min for winter (diamonds) and summer (squares) acclimated *Chaetodipus penicillatus sobrinus* across a range of temperatures.

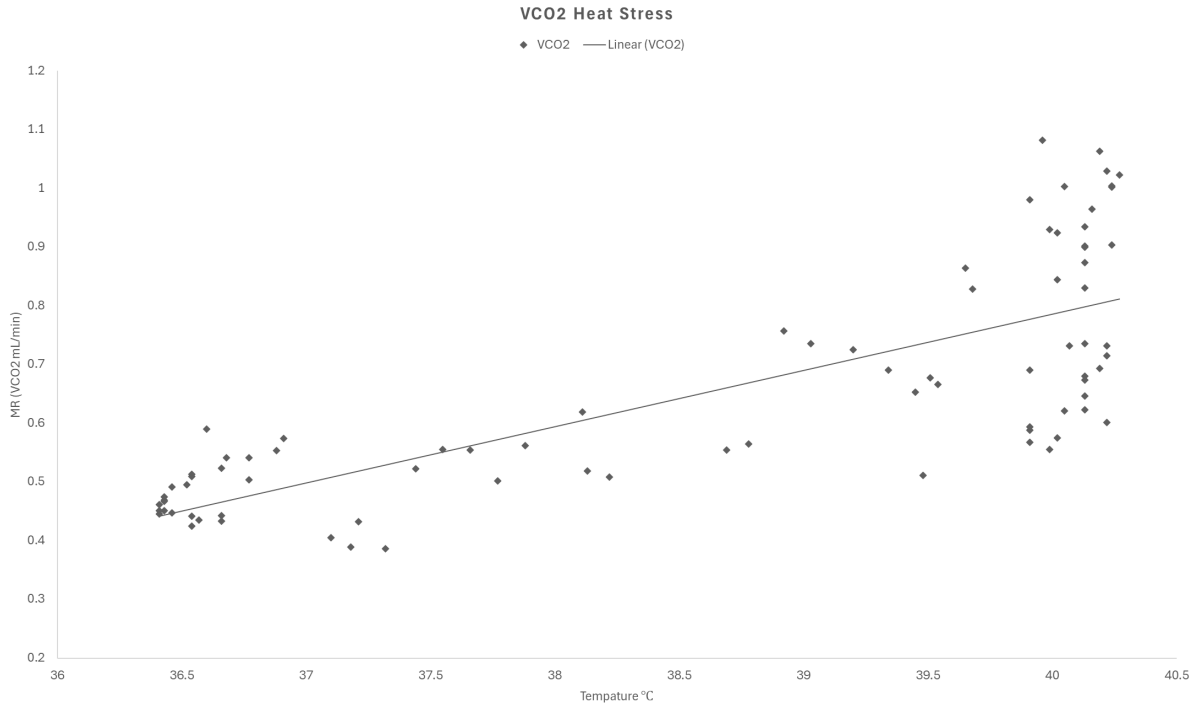

Figure 3. Metabolic rate of a summer acclimated animal going through heat stress, shown as VCO2 over a range of body temperatures. The animal saw its metabolic rate increase as its body temperature increased.

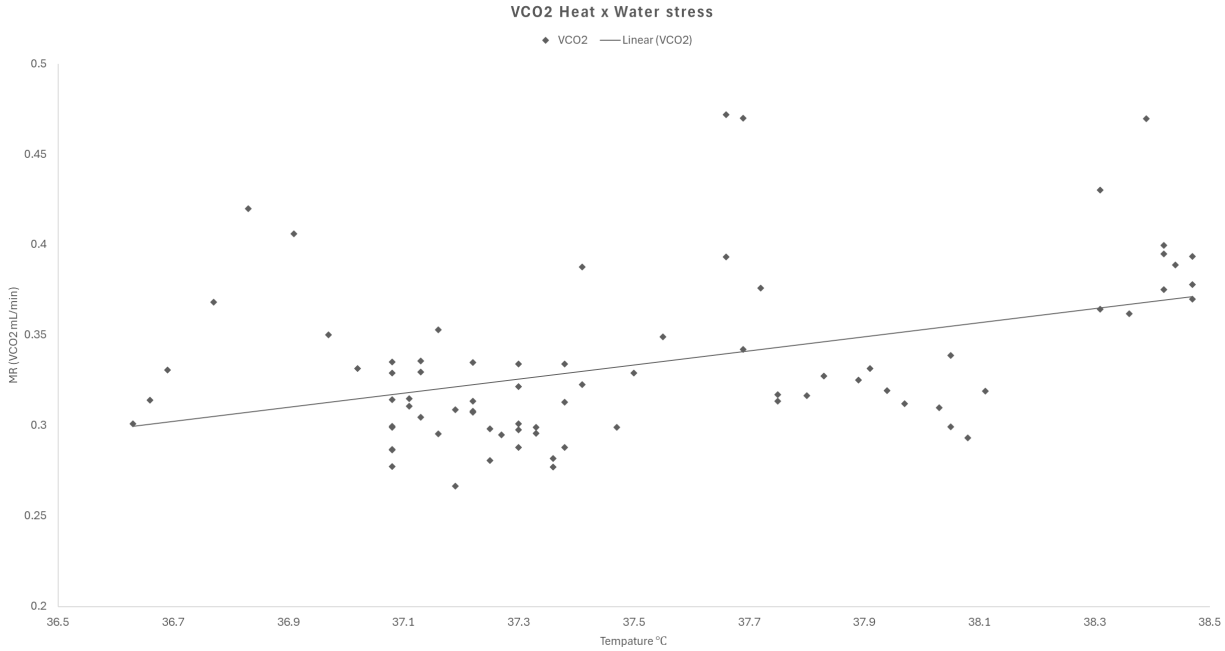

Figure 4. Metabolic rate of a summer acclimated animal going through heat and water stress, shown as VCO2 over a range of body temperatures. The animal saw its metabolic rate increase as its body temperature increased.

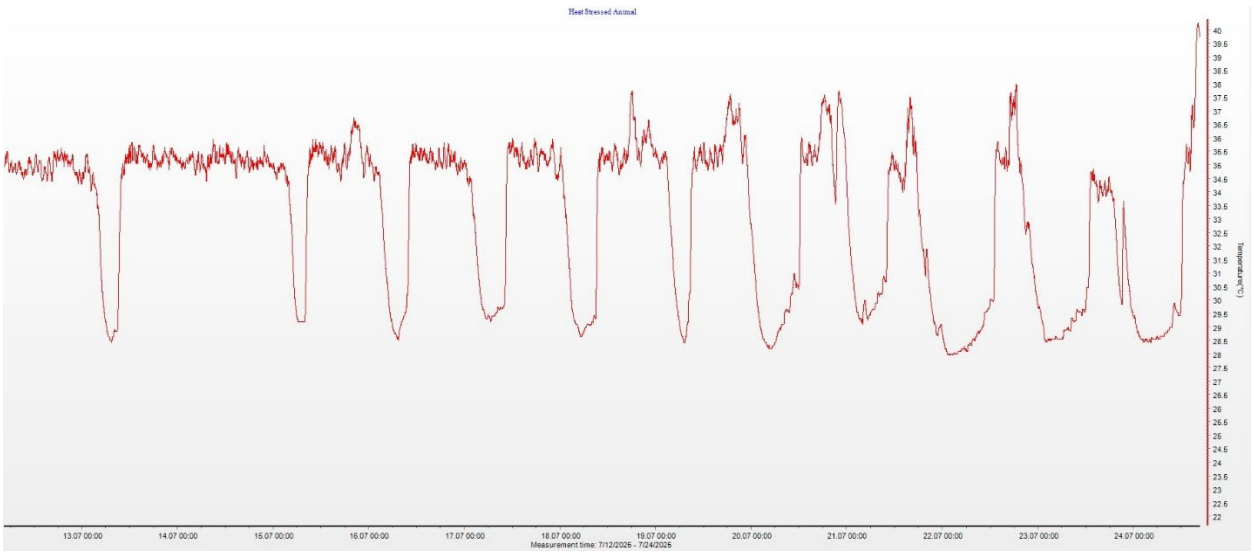

Figure 5. Example of a typical body temperature trace from an animal across two weeks. Note hyperthermia on the right side of the graph during temperature treatment.

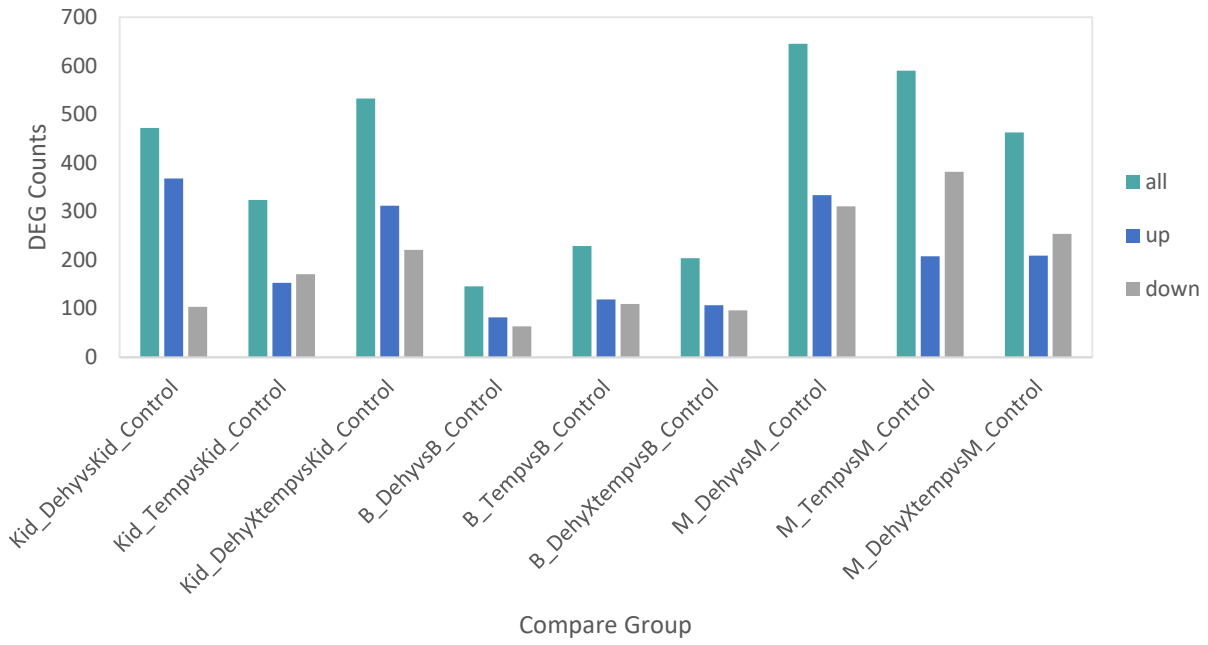

Figure 6. Differential gene expression counts from different tissues (Kid=Kidney, B=Brain, M=Muscle) for each treatment (Temp=heat stress, Dehy=water stress, DehyXtemp= heat and water stress) compared to control group. Histograms are color coded to show up regulated (Up), down regulated (Down), or All differentially expressed gene (DEG) counts.

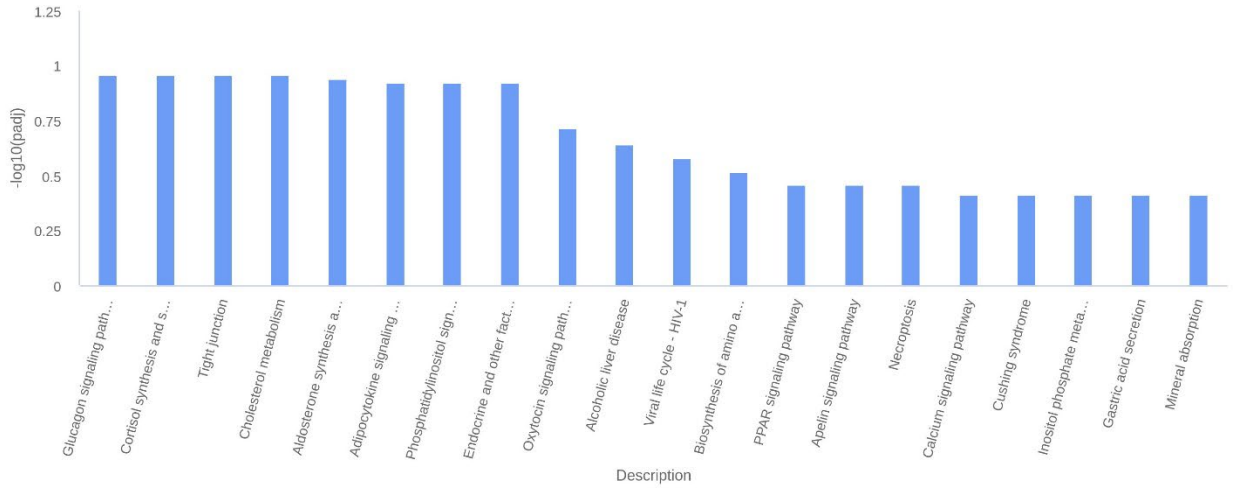

Figure 7. KEGG enrichment analysis plot of differential gene expression of Kidney tissue between Control and Dehydrated groups.

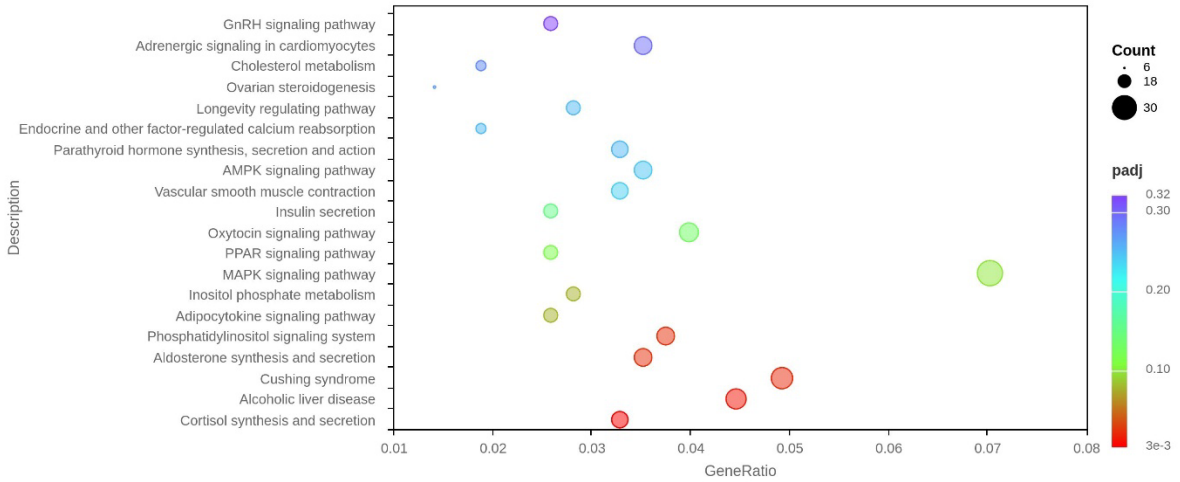

Figure 8. Scatterplot of KEGG enrichment analysis of upregulated gene expression of Kidney tissue between Control and Dehydrated groups.

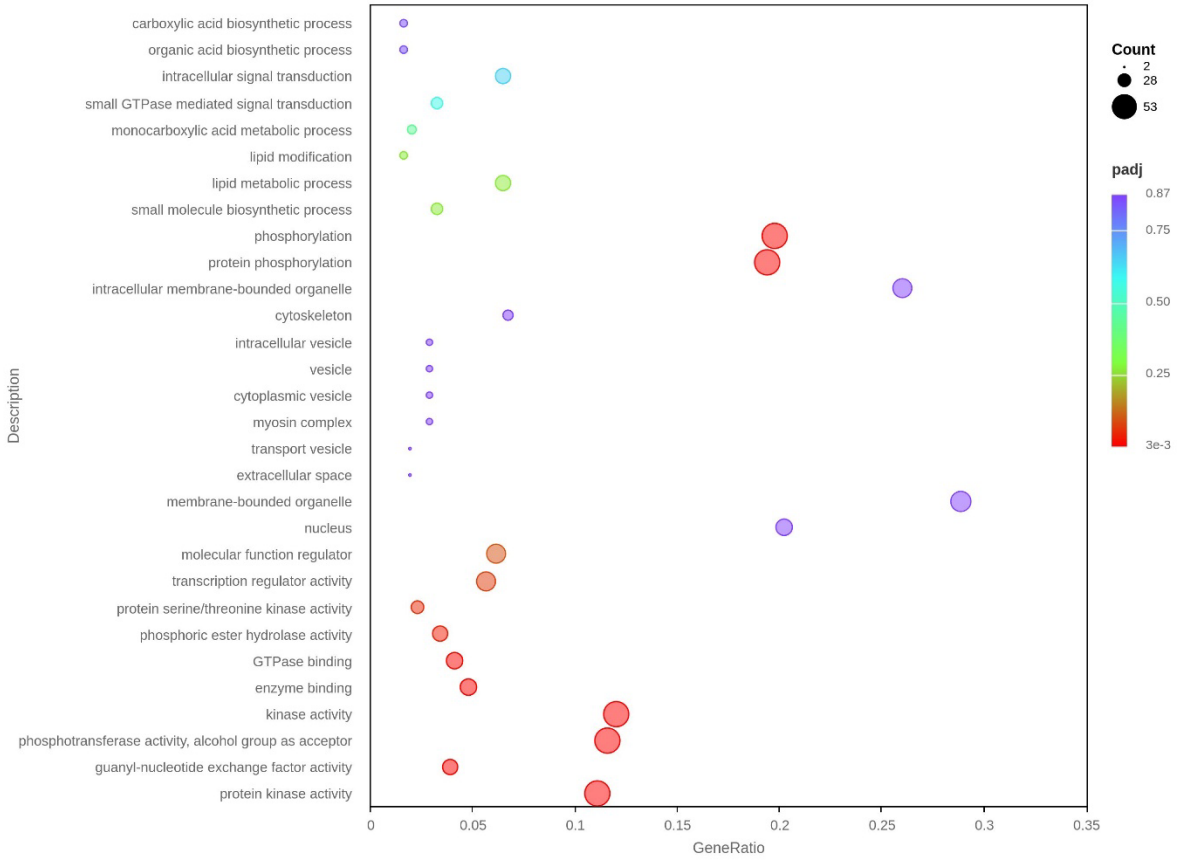

Figure 9. Scatter plot of upregulated pathways from the GO enrichment analysis of Kidney tissue comparing Control and Dehydrated groups.

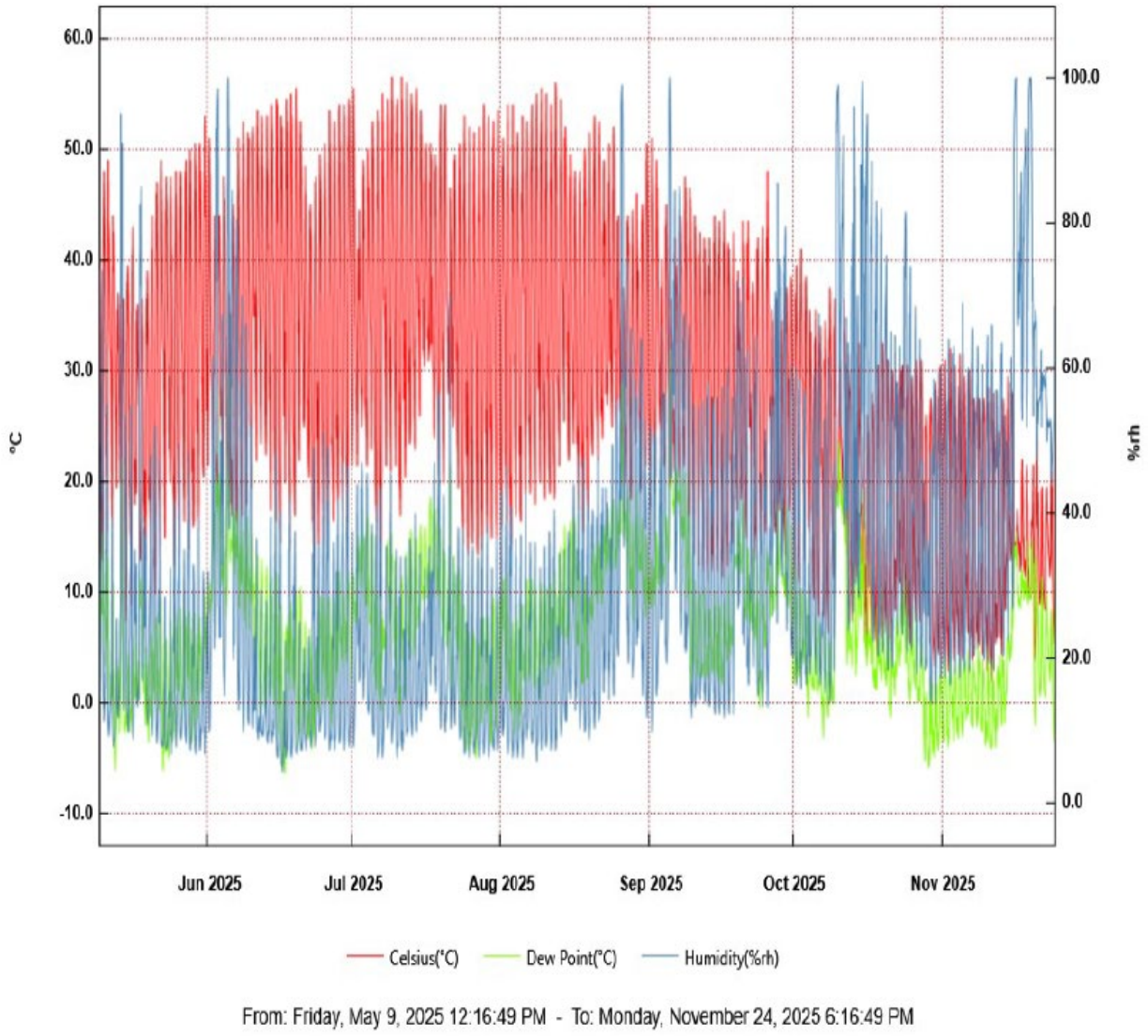

Figure 10. Temperature and Relative Humidity data from logger deployed at ground level in the shade of arrow weed.

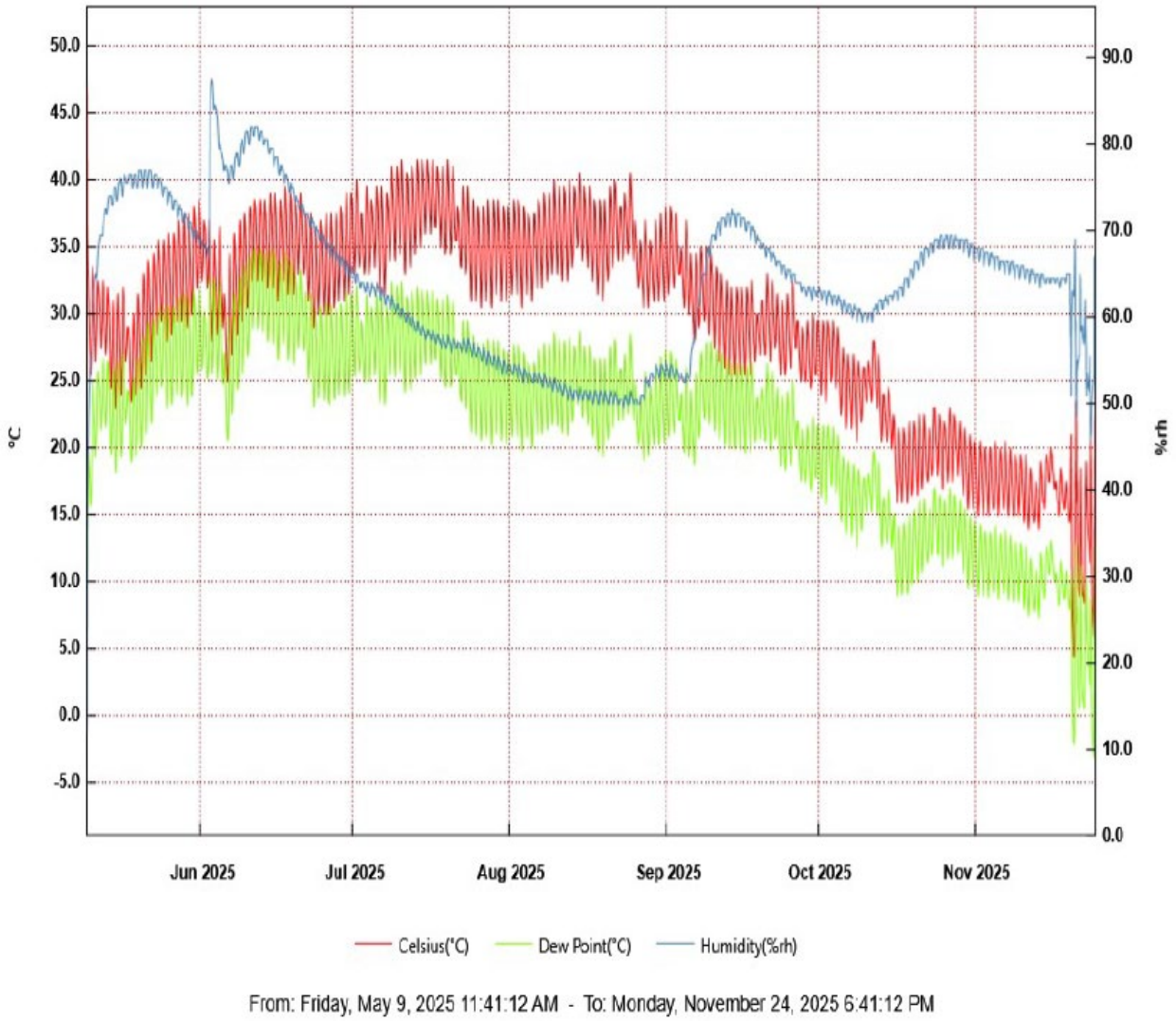

Figure 11. Temperature and Relative Humidity data from logger deployed 20cm underground in open barren sand.

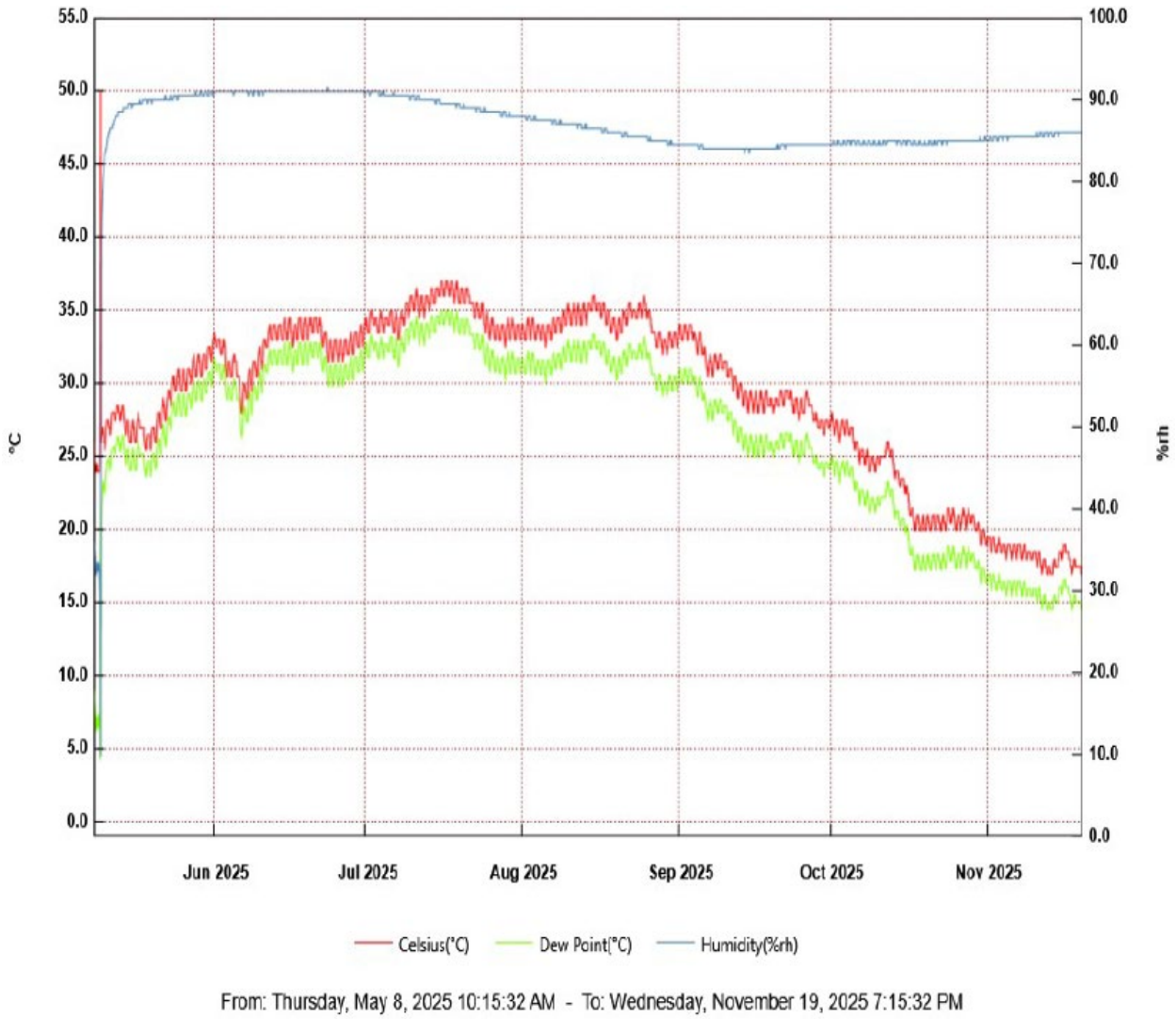

Figure 12. Temperature and Relative Humidity data from logger deployed 50cm underground in open barren sand.

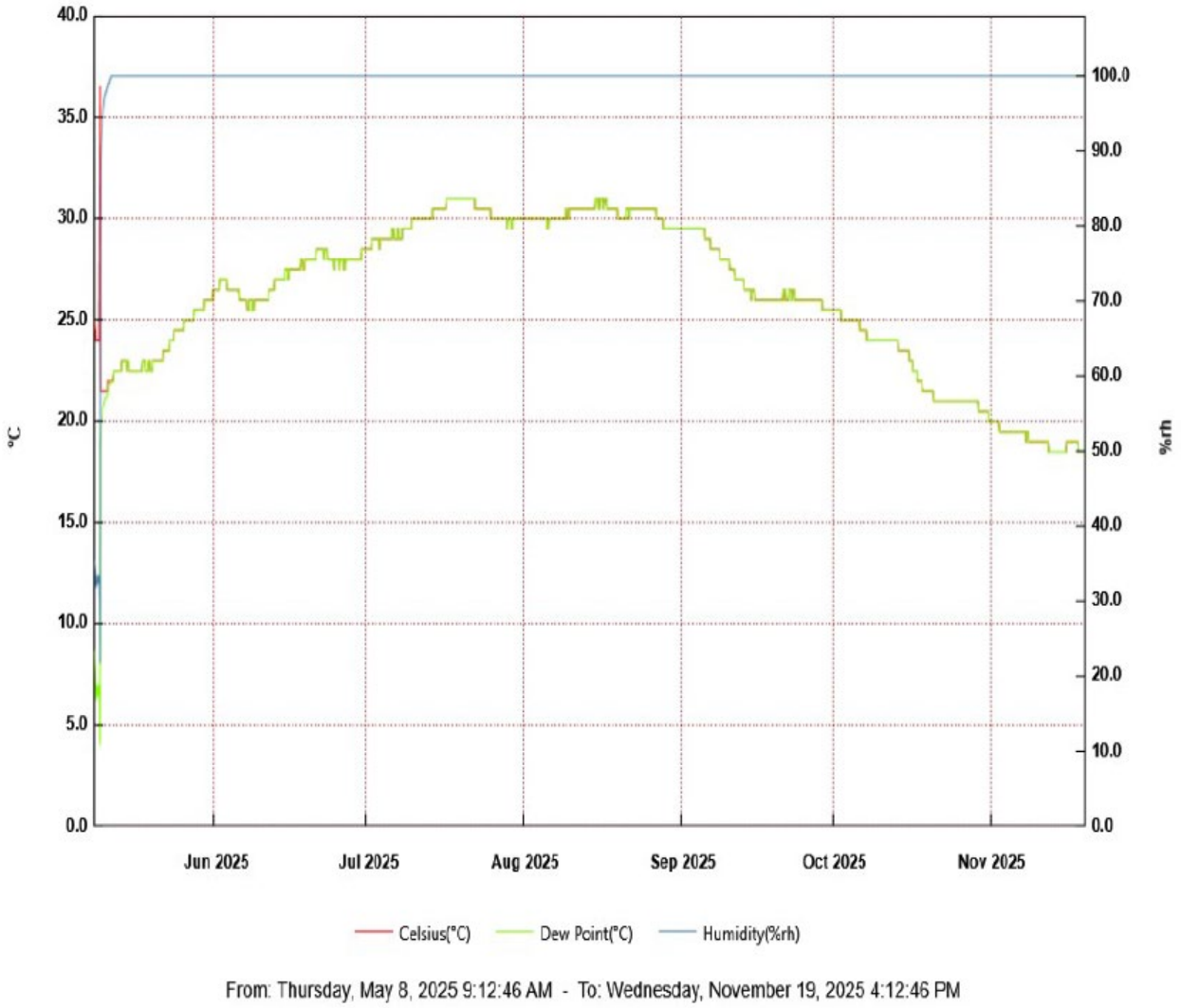

Figure 13. Temperature and Relative Humidity data from logger deployed 50cm underground beneath arrow weed.

APPENDIX A
Citations

LITERATURE CITED

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