**RATIONAL FOR USE OF ANIMALS**: No alternative system can adequately model the complexity of a species’ adaptive repertoire, in particular the complexity and unpredictability of interactions between environmental conditions and individual phenotype (and underlying genotype). For this reasons, efforts to understand how desert-adapted animals survive despite extrinsic water unavailability must necessarily employ live animals. The animals in question are captive raised individuals originating from a wild sample of 12 individuals captured in Arizona several years ago. They are currently maintained in a breeding colony at UNH. If additional mice are required, they are commercially available at the Peromyscus Genetic Stock Center (University of South Carolina).

**RATIONAL FOR CHOICE OF SPECIES AND NUMBERS**: The choice of study species for this project is dictated by the naturally occurring patterns of adaptation (e.g. not all animals are adapted to desert life). The species (*Peromyscus eremicus*) in this protocol have been selected because they exhibit conceptually important patterns of adaptation, studies of which will yield significant new insights into how genetics underlie a biomedically important phenotype. The number of individuals per species to be included in this study represents careful consideration of the ability to process the resultant data, and of the power of analyses to yield meaningful results. Finally, all procedures described in this proposal are constantly evaluated to confirm that they do not produce unnecessary suffering for animals, and yield high quality data.

Aim1 will include 20 adult individuals (*Peromyscus eremicus*, approximately 10M and 10F) in each of 6 treatment groups (n=120 total). Aim 2 will include five juvenile mice (*Peromyscus eremicus*, approximately 10M and 10F) per treatment for each of 6 treatments, multiplied by 4 different time-points (n=120 juvenile animals). The ages of the juvenile mice range from fetal (close to fully developed), to post-weaning, which is approximately 20 days in age.

**Veterinary Care**: All procedures involving live animals will be conducted in the UNH USA approved Animal Resource office, under the supervision of a staff veterinarian. The vet staff and vet technician perform daily checks to ensure animals are healthy. Of note all animal procedures have been approved by the UNH IACUC and are in accord with both The American Society of Mammalogists and the Panel on Euthanasia of the American Veterinary Medical Association (AVMA) guidelines on euthanasia.

**Animal Procedures including Euthanasia:**

1. Animals are housed in standard mouse tubs, and given water in accordance with the experimental plan (either water *ad lib* or water restriction). They are fed desiccated rodent chow. Animals are housed in same-sex pairs except for the purposes of breeding. During experiments that consist of hot and/or dry conditions, care will be taken to maintain experimental fidelity- animals will not be exposed to moist air or cool temperatures.
2. Any procedure that may be accompanied by more than momentary and minor pain (e.g. PIT tag placement) will be conducted under light Isoflurane anesthesia and with supervision by the veterinary staff. Because handling induces stress, which may increase metabolic rates and this water use, animals will be, generally, left undisturbed during experimentation.
3. Weights will be collected in the desert chamber using a standard micro-balance. This procedure does involved handling animals, but seems to be relatively non-stressful. Urine will be collected using a standard (and simple) method. Given mice tend to urinate when handled, they will be quickly picked up, and placed on a surface covered by cellophane. Urine, when present, will be collected using a pipette and analyzed on the urine refractometer. To minimize handling, urine and feces will be collected simultaneous with weighing.
4. Metabolic testing will be accomplished via use of a standard metabolic chamber. The system is modified to enable use in hot and dry conditions, but these modification do not alter the chamber within which animals are held.
5. Desert conditions will be replicated by use of desert environmental chamber. The chamber is walk-in sized, and has been manufactured to hold heat and humidity to user-determined levels. The chamber will produce a diurnal pattern of temperature and humidity, typical of the desert southwest.
6. Euthanasia: In brief, animals are euthanized with Isoflurane overdose followed by decapitation. Within minutes of death, trunk blood is collected and placed into a heparinized vile, and organ tissue is placed into RNAlater filled cryosafe microcentrifige tubes. These tubes are immediately flash frozen in Liquid nitrogen, then transferred to a -80C freezer.