

Utilization of a Native Hibernator in Advanced Physiology Courses.

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Abstract

Concepts related to metabolism and hibernation can be difficult to directly explore in an animal physiology teaching lab. We are developing laboratory protocols that utilize a native hibernator, the thirteen-lined ground squirrel (*Spermophilus tridecemlineatus*). This animal model is well-suited for incorporation into a semester long animal physiology course because of the seasonal changes in metabolism associated with daily torpor and hibernation. For instance, measuring gas exchange in unrestrained animals, we are able to demonstrate significant ($P < .05$) reductions in metabolism between late summer and early fall (35.46 ± 4.35 ml O_2 /kg/min vs 15.71 ± 2.17 ml O_2 /kg/min, mean \pm SE, summer vs fall, respectively, $n=6$) as animals increase their level of torpidity. Similarly, we can examine the changes in substrate utilization during this time period through the measurement of respiratory exchange ratios ($.90 \pm .07$ vs $.78 \pm .07$, summer vs fall, respectively, $P < .05$). Additionally, we will be able to measure and compare ventilatory variables such as tidal volume and minute ventilation in these same animals. Plans are underway to continue data collection and the development of teaching protocols once animals have reached their full hibernation state. The thirteen-lined ground squirrel is an abundant, easy to care for native hibernating species that can be used to greatly enhance the educational quality of advanced physiology labs.

Introduction- Laboratory Challenges

- Metabolism is an area of physiology that can be explored in a laboratory setting.
- Whereas the measurement of oxygen consumption (VO_2) can be conducted in rats or human subjects, the metabolic changes associated with hibernation are most often inferred.
- Thus, we aim to develop laboratory procedures to allow for the direct observation of metabolic alterations in a hibernating species.

Introduction- Thirteen Lined Ground Squirrel Metabolism

- The thirteen-lined ground squirrel (*Spermophilus tridecemlineatus*) is a native hibernator that shows remarkable seasonal changes in metabolism.
- These animals show peak activity in the summer months, and transition to winter hibernation with repeated torpid bouts during the early fall.
- These animals are easy to capture and maintain and can thus be an excellent animal model.

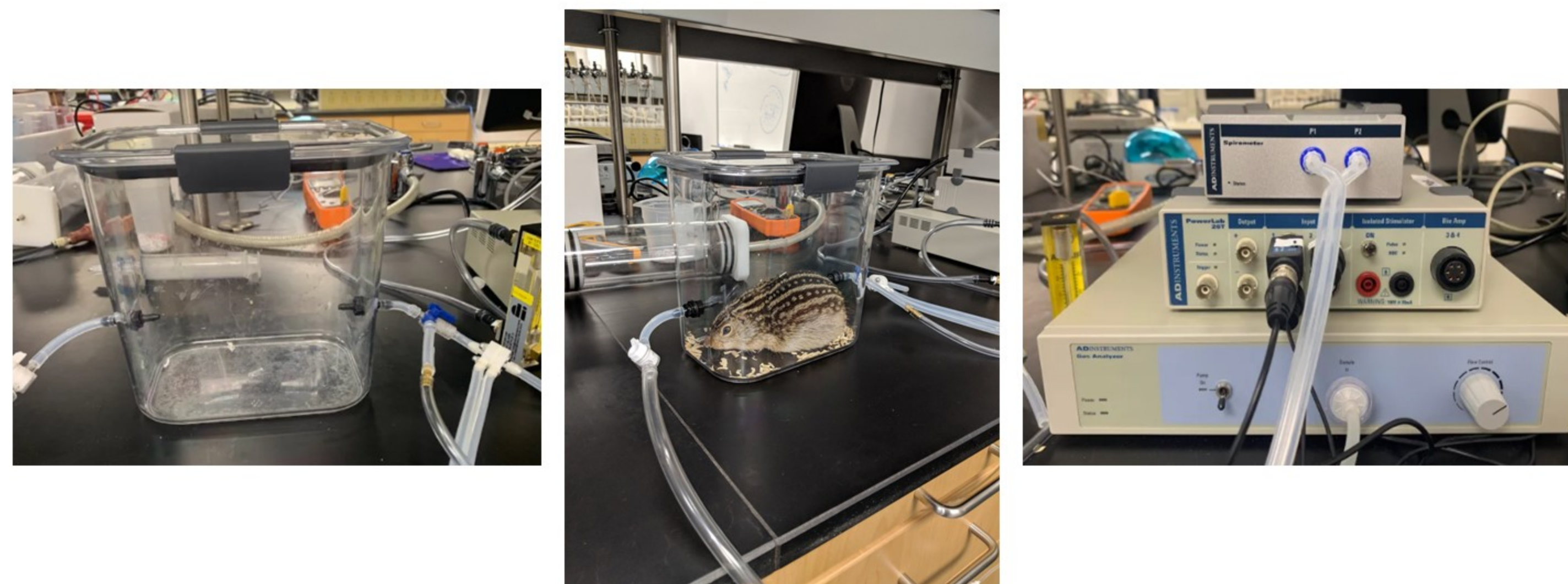


Methods- Capture and Care

- All procedures were reviewed and approved by the Luther College Institutional Animal Care and Use Committee.
- The capture and utilization of thirteen-lined ground squirrels in teaching and research requires approval by both the Iowa Department of Natural Resources and the U.S Department of Agriculture.
- Squirrels were captured throughout the summer months and maintained in a dedicated room of the Luther College Live Animal Facility.

Methods- Metabolism Measurements

- VO_2 was measured with open (flow-through) respirometry (ADInstruments Power Lab 26t, Gas Analyzer, and Spirometer)
- Metabolism, respiratory exchange ratio (RER) and lung volumes were calculated using modified rat metabolism protocols provided by ADI.
- Squirrels were studied at three different time points.
 - Summer (Active, July)
 - Fall (Pre-hibernation, September)
 - Late Fall/Early Winter (Hibernation, November)
- Animals were placed in a custom-built hibernation chamber by the third week of October.
 - Continuous airflow is provided through the chamber
 - Temperature is maintained at $4^\circ C \pm 0.5^\circ C$



Results

Ground squirrels show profound metabolic and respiratory changes between the summer and pre-hibernation states.

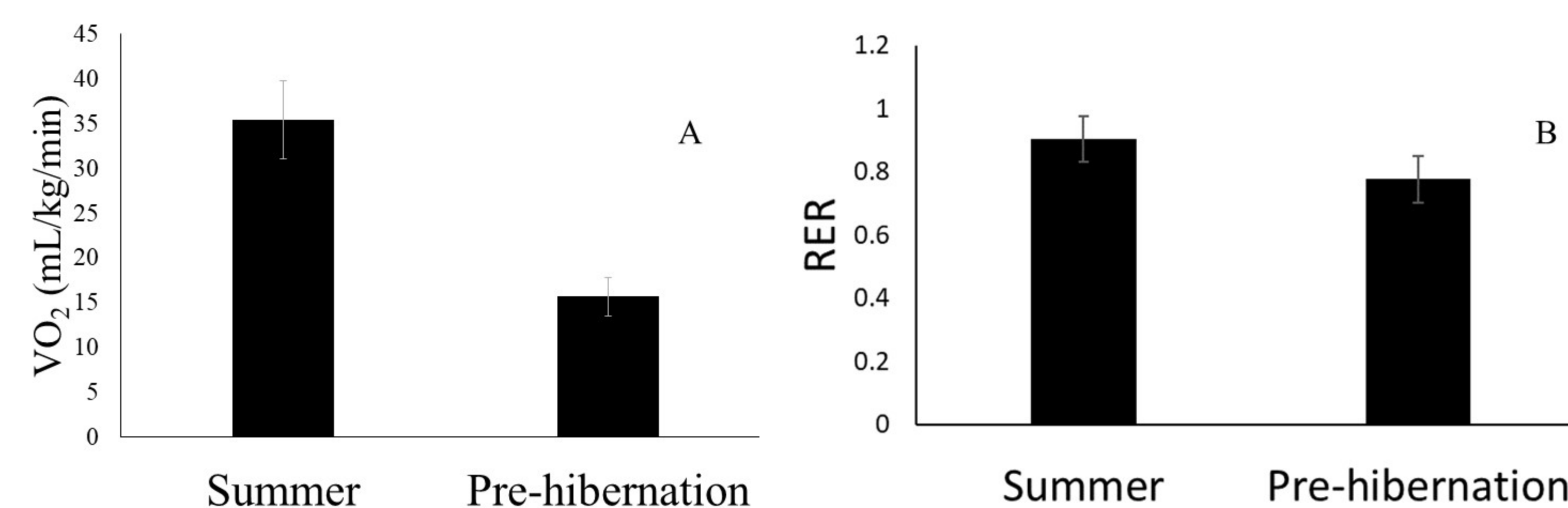


Figure 1. Both oxygen consumption (VO_2 , panel A) and respiratory exchange ratio (RER, panel B) are significantly reduced during the fall transition state. * $P < .05$ vs summer, $n=6$.

Table 1. Ventilatory parameters show seasonal alterations.

Squirrel	Time Frame	Tidal Volume (mL)	Respiratory Rate (breaths/minute)	Minute Ventilation (mL /minute)
2021-5	summer	0.66	220	145.2
2021-5	pre-hibernation	0.182	184	33.49
2021-5	hibernation	0.102	30	3.06

Results continued

- Thirteen-lined ground squirrels can be easily captured and maintained in a live animal facility.
- Only slight modifications to equipment and protocols are necessary to study these animals in a laboratory setting.
- These ground squirrels show significant alterations in ventilatory and metabolic parameters between the active, pre-hibernation and hibernation phases that are in agreement with published findings from the same and similar species (Heldmaier, et al., and Milsom and Jackson).



Discussion- Laboratory Technique

- The measurement of metabolism in a teaching lab setting can greatly enhance physiology instruction.
- Whereas the use of humans or common laboratory animals such as rats is beneficial to the study of metabolism, the use of a hibernating species provides opportunities to expand the exploration of metabolism.
- The thirteen-lined ground squirrel is a native hibernator that can be successfully maintained in a live animal facility.
- The thirteen-lined ground squirrel displays metabolic and ventilatory changes over the course of several months, and as such could be studied multiple times in a semester-long course.
- The profound changes in VO_2 and RER shown by this ground squirrel provide opportunities to examine the concepts of heat production, energy expenditure and metabolic substrate utilization during active, pre-hibernation, and hibernation phases of the year.

References:

- Heldmaier, G., Ortman, S., and Elvert, R., 2004. Respiratory Physiology and Neurobiology 141, 317-329.
- Milsom, W.K., and Jackson, D.C., 2011. Comprehensive Physiology 1, 397-420.