Introduction

At the doctor's office, the doctor's team always begins by measuring body mass and temperature. It is not surprising that these two physiological parameters are the most important indicators of one's health. Body temperature is the best indicator of whether or not there is an infection, and body mass provides insight into the quality of a person's diet. For most adult mammals, these vital signs are relatively stable. Mammals that hibernate, however, are an exception and provide an interesting way for students to explore how warming climatic temperatures affect the ability of these mammals to maintain their optimal balance between body temperature and mass.

Yellow-bellied marmots (Marmota flaviventris), golden-mantled ground squirrels (Callospermophilus lateralis), and black bears (Ursus americanus) are able to reduce metabolic rate and lower internal body temperature in response to the cold ambient temperatures experienced during winter hibernation. When they drop body temperature to near ambient temperature, we say they are “hibernating.” This allows for energy conservation during a period when food availability decreases drastically in the winter season and mammals that hibernate cease food intake. Preparation for the winter in mammalian hibernators triggers neural, hormonal, and metabolic changes that promote survival throughout periods of virtually no environmental food and extreme ambient temperatures.

Precise coordination between many organs is required to maintain homeostasis for the duration of the hibernation season (September–April). In recent years, important relationships between bone, fat, reproductive, and brain tissues in mammals have come to light; all four systems share interconnected regulatory mechanisms of energy metabolism. Understanding the biological mechanisms by which hibernators can survive the prolonged fasting and disuse of muscle and bone associated with these extreme environmental challenges will provide critical information regarding the limit of overlap in mammalian systems and of skeletal plasticity, and may contribute valuable insight into human medical challenges, such as weight regulation and bone loss. As such, teaching students early on about the physiological changes that can occur during hibernation may stimulate interest in the importance of their own metabolism and how it relates to food intake, body-weight regulation, and bone loss.

“Secrets of the Hibernators,” like all of the self-guided inquiry-based kits developed by the Natural Sciences Education and Outreach Center (NSEOFC) at Colorado State University (CSU), represents a collaboration between research scientists/engineers and educational specialists. The kits are designed to make major scientific concepts related to university research accessible to secondary-level students. Connecting students to data and materials provided by scientists not only helps address state and national educational standards, but also allows students to explore concepts that are important to scientists (e.g., AAAS, 1994; NGSS Lead States, 2013) but often neglected by developers of mass-market curricular materials.

Four student-led investigations are packaged and woven together to show students how biologists study hibernators to help solve human problems. Activity 1 has students graph the body mass of a yellow-bellied marmot for each month of the year. Students use...
the graph to answer questions about the seasonality of energy storage. Activity 2 explores data from a hibernation temperature experiment involving two groups of golden-mantled ground squirrels. Students use the data to determine which group of squirrels were put in a warmer environment halfway through their hibernation. In activity 3, students use a model system involving yeast to duplicate the squirrel experiment, which allows students to collect and share their own data. The final activity involves the study of two microscope slides, one from a young black bear and the other from an old black bear, to observe the differences in bone density. Students compare how age affects bone density in hibernating mammals versus humans.

The kit is designed to work at multiple grade levels. It has been successfully used with sixth-graders and up. Table 1 lists the Next Generation Science Standards that are addressed.

Table 1. Next Generation Science Standards addressed.

| Activity | Scientific Practices                                                                 | Crosscutting Concepts                  | Disciplinary Core Ideas                                                                 |
|----------|---------------------------------------------------------------------------------------|----------------------------------------|-----------------------------------------------------------------------------------------|
| 1        | Analyzing and interpreting data; constructing explanations                            | Patterns; cause and effect              | LS1.B: Growth and Development of Organisms                                              |
|          |                                                                                      |                                        | LS2.A: Interdependent Relationships in Ecosystems                                        |
| 2        | Analyzing and interpreting data; constructing explanations                            | Patterns; cause and effect              | LS1.C: Organization for Matter and Energy Flow in Organisms                              |
| 3        | Developing and using models; analyzing and interpreting data; engaging in argument from evidence | Patterns; cause and effect; systems and system models | LS1.C: Organization for Matter and Energy Flow in Organisms                              |
| 4        | Developing and using models, analyzing and interpreting data; obtaining, evaluating, and communicating information | Patterns; scale, proportion, and quantity; structure and function | LS1.A: Structure and Function                                                            |
|          |                                                                                      |                                        | LS1.B: Growth and Development of Organisms                                              |

Table 2. Supplies necessary for a single kit.

| Quantity | Item                                           | Source                                                                 |
|----------|------------------------------------------------|-----------------------------------------------------------------------|
| 1        | Rugged plastic case                            | Pelican 1150                                                          |
| 1        | Instruction booklet                            | Downloadable PDF file: https://www.cns-eoc.colostate.edu/stem-kits/secrets-of-the-hibernators/ |
| 1        | Digital thermometer                            | Scientific educational supply                                         |
| 3        | Pipettes                                       | Scientific educational supply                                         |
| 3        | 50 mL beakers                                  | Scientific educational supply                                         |
| 1        | Metal scoop                                    | Scientific educational supply                                         |
| 1        | "Viney" apparatus                              | 3D-printed .STL file can be downloaded: https://www.cns-eoc.colostate.edu/stem-kits/secrets-of-the-hibernators/ |
| 1        | Quick rise yeast and sugar                     | Grocery store                                                         |
| 1        | Hot and ice water                              | Tap water and ice                                                     |
| 1        | Bromothymol blue indicator 0.03%               | Scientific educational supply                                         |
| 1        | Stopwatch                                      | Scientific educational supply                                         |
| 2        | Bear bone microscope slides (protected in a sturdy acrylic mount) | These were custom made at CSU; downloadable micrographs: https://www.cns-eoc.colostate.edu/stem-kits/secrets-of-the-hibernators/ |

○ Materials

“Secrets of the Hibernators” kits are housed in 15 durable Pelican brand cases that contain the materials necessary for pairs of students to complete the self-guided inquiry within a single class period (Figure 1). The kits use a combination of locally sourced items, 3D-printed models, and materials that can be purchased from scientific educational supply companies (Table 2).

○ Investigations

Before students start their activities, they copy the following research question into their notebook: *Will the lives of hibernators be easier or more difficult in a warming climate?*
Activity 1: Seasonal Changes in Yellow-Bellied Marmot Body Mass

Students graph and interpret data, tracking changes in the seasonal body mass of yellow-bellied marmots collected by CSU researchers. Students use their graph to infer relationships between eating, using, and storing the energy in food throughout the year. These relationships reveal the evolutionary adaptations in marmots’ metabolism that allow them to survive in an environment that produces little or no food for nine months of the year. Students use their graph to predict when the marmot begins its hibernation. Marmots and humans are both mammals, yet the adaptive life style of the marmot – which includes periods of consuming large numbers of calories followed by long periods of inactivity and fasting – is not necessarily healthy for a human. However, learning how the marmot regulates its metabolism throughout the year may provide information that could help humans with certain challenges like obesity and osteoporosis.

Activity 2: Hibernating Temperatures for Golden-Mantled Ground Squirrels

In the second activity, students use data collected by scientists to see what would happen to the body mass of an animal hibernating at a warmer-than-normal ambient temperature. During the experiment, two groups of ground squirrels were placed in identical lab environments in June. In October, one group was placed in a 5°C environment that mimics their normal hibernating temperature while a second group was placed in a warmer, 22°C environment (Florant et al., 2012). Students graph and interpret the body-mass data for the two groups of squirrels collected by scientists during the experiment (Figure 2). At this point in the activity, it is not revealed to students which group represents hibernating at the normal temperature and which group was hibernating at a warmer temperature. The data are set aside to be reflected upon after the third activity.

Activity 3: Using Yeast to Explore the Effect of Temperature on Metabolic Rates

In activity 3, students perform a modified version of the ground squirrel experiment using yeast as a model system to explore how the metabolism of hibernators can change with seasonal temperature changes. Yeast is a unicellular fungus and very different from multicellular mammals but has been used as a model system in aging studies (Jarolim et al., 2004). Furthermore, yeast has a metabolic rate similar to that of mammals when it is at our average body temperature of 37°C (98.6°F). Although yeast do not hibernate, they provide a convenient and safe way to explore how metabolism can be affected by changes in temperature.

Students begin by placing two pipettes filled with activated yeast solution into wells filled with warm tap water (37°C). The stems of the pipettes are placed in solutions containing bromothymol blue (BTB) indicator solution. A third well, without a pipette stem, is filled with BTB as a color control. Students keep track of

Figure 1. The “Secrets of the Hibernators” kit is housed in durable cases for safe transport and many years of reuse. (Photo by Sam Harsh)

Figure 2. Science notebook entry for Activity 2.
and record the number of carbon dioxide bubbles that emerge from the pipettes over a period of five minutes. In preparation for the second experiment, the warm water is removed from the wells. In the second experiment, one well is filled with fresh warm tap water (37°C) while the other is filled with ice water (near 0°C). Once again, students keep track of and record the number of bubbles emerging from the pipette stems (Figure 3).

Each pair of students share their quantitative data in a class chart so that the average number of CO$_2$ bubbles produced in each experiment can be calculated. Students are also asked to describe the color changes (qualitative data) observed in the wells filled with BTB. Students are now asked to relate the metabolic changes, as measured by CO$_2$ production in the yeast solutions at different temperatures, to the graph comparing body-mass changes in the ground squirrels hibernating at two different temperatures. Hibernators must store enough food and use it at a slow enough rate to survive the winter. Students are asked to use their data from activity 2 to infer whether both groups would be able to survive the winter.

Activity 4: Comparing the Bone Structure of Old & Young Black Bears

Bears are the largest animals that hibernate. One of the mysteries of hibernators is how they maintain their bone strength without being active for half the year. Inactivity in humans can lead to bone loss. In fact, human bones must experience the stress from exercise to maintain their strength. Researchers study the bones of hibernating animals to better understand how they maintain strength even during periods of inactivity (McGee-Lawrence et al., 2008). In this activity, students use a microscope and thin sections of cortical bone prepared from the femurs of black bears to make observations about the bones of young and old bears. Images from these slides can be accessed on our website (Figure 4). Students draw, label, color, and make observations without being told the age of the bears represented by the two slides. After students complete their drawings, they are told which came from a young bear and which came from an old bear. The bone density of hibernating bears is maintained during inactivity and actually increases with age because the porosity decreases during hibernation and with age (McGee-Lawrence et al., 2009). This is unique, and opposite to what happens in humans. Humans lose bone mass during inactivity and may develop the bone-loss condition known as osteoporosis as they age, because human bone becomes more porous with inactivity and aging.

Researchers at CSU compared the proteins in the bones of hibernating and non-hibernating marmots to find clues on how they might lower their metabolism and maintain bone density. They found that a protein called monoaglycerol lipase was at a much higher concentration in the bone marrow of hibernating marmots than in summer marmots (Mulawa et al., 2018). Interestingly, this protein plays a role in metabolism.

Interview with a Student

The instruction booklets for kits developed at the NSEOC each conclude with an interview of a CSU student from a population traditionally underrepresented in the sciences. The hope is that their story will help inspire students to think about pursuing careers in STEM fields. The interview questions reveal how they became interested in science, who their favorite science teacher was, whether or not they did science fair projects, and what their current research at CSU is about. For this booklet, we featured a female student who was interested in becoming a veterinarian but got hooked on studying marmots through an undergraduate research opportunity in the Florant lab.

Evaluation of Effectiveness

The STEM Center at CSU conducted the independent evaluation for the “Secrets of the Hibernators” self-guided student science kit. The evaluation was intended to serve two objectives: (1) to provide feedback for iterative development and refinement of the education kits, and (2) to collect participant data and provide results to gauge the success of the kits.

“Secrets of the Hibernators” was pilot tested among both middle and high school age groups at the NSEOC lab space at CSU. To meet the first evaluation objective, the evaluator conducted structured observations of the on-site kit implementations. Across all groups, participants were able to use and handle the equipment safely and remain engaged, interested, and on task throughout. Based on the structured observations across student groups, several iterative changes were made to the activities and instructions to more closely reflect actual research being conducted at CSU, and to prioritize focus on concepts that students would likely not be exposed to in their own science classrooms. To meet the second evaluation objective, the evaluator conducted student notebook review among a sample of five to eight student participants in each group.
pilot group. Based on previously validated assessment and scoring methodologies (Ruiz-Primo et al., 2004), notebook assessments documented completeness, clarity, organization, and communication (Performance), as well as activity implementation (Opportunity to Learn). Across groups, students demonstrated the ability to accurately execute math calculations, complete drawings and graphs with appropriate labels, and provide written answers to questions in the instruction booklet that demonstrated an ability to apply concepts learned. Instructor-facilitated group discussions after each implementation corroborated students’ ability to understand how educational goals fit together conceptually after completing the kit activities. By the final iteration of the instruction booklet, notebook assessments indicated that high school students were most likely to finish all four activities, and early middle school students were most likely to finish three activities.

Although a formal assessment of student and teacher satisfaction with the kits has not yet been conducted, observations and notebook assessments conducted during pilot testing indicate that students were engaged in and enthusiastic about the activities, were able to learn or better understand the scientific concepts, and were able to complete the kit activities with minimal guidance. It is thus expected that teachers can successfully implement the kits in their classrooms to supplement their own curricula. The kit is designed such that activities can be divided up and completed in separate sessions, so that students across age groups and experience levels can finish all four activities. An online instructor implementation form will accompany kits that travel to Colorado schools in order to identify teachers’ interest in, expectations of, and satisfaction with the kits as well as barriers and facilitators to implementation. These additional data will help measure the success of the kits on a large scale and identify any additional helpful adaptations for various age groups.

**Conclusion**

The “Secrets of the Hibernators” kit represents a collaboration between university scientists and educational specialists with the aim of connecting secondary-level students to exciting and inspiring current research. The self-guided inquiry-based kit invites students to participate in the process of science by exploring natural patterns. The scientific participation takes the form of plotting and interpreting data, making predictions, collecting and sharing experimental data with student colleagues, creating artwork to make accurate observations, and applying newfound knowledge to written responses. These are essential elements of the scientific process. It is our hope that interweaving real hibernator research data with student-conducted experiments and observations is a more engaging way to learn about food intake, body mass, metabolism, exercise, and bone strength. Additionally, the knowledge gained by students during these activities allows them to infer the consequences climate change might have for our fellow hibernator mammals adapted to living in extreme environments.

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