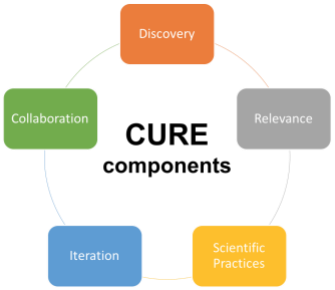
Lab 5 – Thermal Exchange



Principal Investigator(s)**: Jennifer Forbey**
Protocol revised by **JF 08 October 2019**

Objective
The purpose of this study is to:
1. Understand the **relevancy** of temperature to understand the physiology of animals
2. Use **scientific practices** to quantify and compare how morphology influences thermal exchange with the environment
3. Understand how the environment and animal morphology influences the physiological process of thermal balance and exchange
4. Develop protocols for future **iteration, collaboration** and **discovery** of thermal balance in animals.

Preparing study skins: prepared by 2019 class of:

Skinning animals is a useful skill in the biological research field. Proper preservation of animal anatomy and precise dissection skills are essential for creating repeatable and accurate observations of animal anatomy and physiology. By having this skillset, one becomes more employable, being able to increase the sample size of research experiments and maintain well preserved scientific specimens for future research (Winker). In this case, dissecting various ptarmigan skins allows us to observe the unique thermodynamic properties of seasonal feathers (Nord). (CHG)

Supplies:
Scalpel
Scissors
Dissection tray
Forceps
Probe
Table mats
Ptarmigan bird (3)
Gloves
Bleach (cleaning)

Procedure:

1.Allow frozen bird to thaw

2.Make ventral incision from base of tail to the neck, parting feathers to reveal skin as you go to preserve as many feathers as possible without cutting them. If breast muscle has been removed, start peeling skin towards armpits, neck, and legs.

3. Make an incision around the circumference of the top of the neck in order to pull the head through the skin.

3. Peel each side of the skin towards the wing, taking the wing off with the skin If possible. Peel as much skin off the wing as possible before breaking the humerus to detach the wing from the body. If necessary, cut through muscle tissue and/or cut the wing entirely off. Save wings if they needed to be cut off of the body.

4. Separate the skin from the rest of the body, working your way towards the back of the bird (dorsal side).

5. Cut both feet off the body at the tibiotarsus and tibiometatarsus joint (ankle). Save for later! Now peel the skin off the legs

6. Keep the tail attached to the skin as well, in a similar fashion to the wings and continue peeling skin up the back of the bird towards the head.

7. Now, pull the skin, wings, and tail up over the head (where you should have made an incision to do this) like you are taking off the bird's skin sweater.

8. Clean up table area disposing of contents in proper biohazard bins and wiping down tables with a 10% bleach solution.

Nord, A. Seasonal variation in thermal responses to changing environmental temperature in the world's northernmost land bird. (2018). Journal of Experimental Biology.
http://apps.webofknowledge.com/full_record.do?product=WOS&search_mode=GeneralSearch&qid=6&SID=5E8YtWNf2nNgNNGrIDk&page=1&doc=
Winker, Kevin. (2000). Obtaining, preserving, and preparing bird specimens. Journal of Field Ornithology. 71. 10.1648/0273-8570-71.2.250.
https://sites.uco.edu/cms/biology/files/Preparing_Ornithological_Specimens_for_UCO.pdf

Preparing thermal models

Prepared by: Amanda Hunter, Amanda Hancock, Kendra Hollar, Jeremiah Sullivan, Natalia Jensen (2019)

Thermoregulation is a process that mammals use to maintain their core internal temperature. Ptarmigan roost in the snow where the temperature exceeds their lower critical temperature (Stokkan, 1992). Ptarmigan have plumage; layers that act as an insulator by trapping pockets of air between the feathers (Tickell, 2003). To mimic this adaptation, we are using PYGs wrapped in ptarmigan skins and ice baths to measure the effect of Ptarmigan's plumage on thermoregulation.

1. Gather necessary equipment:
    a. PPE – gloves, lab coat, goggles
    b. Bird skins
    c. PYGs – to replicate a body cavity
    d. PYG dowel – to plug cavity
    e. PYG eye buttons
    f. Superglue/tape
    g. Ice baths
    h. Ziploc bags
    i. Bleach solution
    j. Paper towels

2. Prepare PYGs by attaching the eye button to the PYG dowel (plug) with tape. Place a long piece of tightly rolled inverted tape over the top of the dowel and attach this tape with another strip of tape around the shaft. Stick the button on the end of the dowel on the inverted tape (should be the sticky side up). Put a small strip over the top of the button as well to completely secure it.
3. Place the PYG dowel (plug) inside the PYG.
4. Place PYG in ice bath with about 4 cm of ice below the PYG.
5. Cover the PYG with ice until completely covered with about 1 cm of ice. Place lid on ice bath.
6. Begin timer for duration of measuring temperature. *See “preparing iButtons” protocol.
7. Leave PYG in ice bath for 15 minutes.
8. Repeat steps 2-7 with all 6 PYGs.
9. Next, we will cover each PYG with a ptarmigan skin. **Make sure to keep track of skins and which bird they belong to.**
10. To cover each PYG, place the skin over the top of the PYG and wrap around the bottom as best as possible. Tuck wings to the sides, and slide feet under the PYG.
11. Place the wrapped PYGs in the ice baths and repeat steps 4-7 for all 6 PYGs.
12. After the 15 minutes, remove PYGs from ice baths.
13. Remove skins from PYGs and remove eye buttons from PYG dowel (plug). Throw away tape in biohazard waste container.
14. Clean PYGs and dowel with 10% bleach solution and wipe down with paper towels until dry. Paper towels used to clean PYGs should be disposed of in biohazard waste container.
15. Wrap used skins around corresponding carcass and place back in original bags for storage.

Citations:

Stokkan, Karl-Arne. “Energetics and Adaptations to Cold in Ptarmigan in Winter.” *Ornis Scandinavica (Scandinavian Journal of Ornithology)*, vol. 23, no. 3, 1992, pp. 366–370. *JSTOR*, www.jstor.org/stable/3676662.

Tickell, W. L. N. “White Plumage.” *Waterbirds: The International Journal of Waterbird Biology*, vol. 26, no. 1, 2003, pp. 1–12. *JSTOR*, www.jstor.org/stable/1522461.

Preparing iButtons (Prepared by: Alyssa, Logan, & Cammi)

The Thermochron iButton is an internal temperature recording system. This device is advantageous to biologists as it allows them to measure the temperature of an organism, terrestrial or marine, over a certain length of time (Virens & Cree 2018). Due to their size, however, they are generally restricted to vertebrates. Thermoregulation, thermal environment, and organism temperature are all areas of research that the Thermochron iButton can be helpful in. In one study, iButtons were attached to ground squirrels and used to measure the difference in body temperature during hibernation between males and females (Gur & Gur 2015).

1. Open 1-wire viewer.jar
2. Insert button into the usb sensor
3. Click on the name that pops up in the device list (–F5); one button at a time so not to get confused.
4. Under the Thermochron tab
    a. mission active = false (if true click ‘disable mission button’)
    b. Click ‘Start New Mission’
        i. Check box for ‘Synchronize Real-Time Clock’
        ii. Set sampling rate to 1 (minute)

iii. Click ‘Okay’ to start mission

5. Wait until mission active status turns to ‘True’ before removing button
6. Use the least amount of tape to attach button to the rod
7. Record time experiment started and stopped (this will be your experimental time)
8. After data collected
9. Pop button back into the usb sensor
10. Click on the device
11. Under Thermochron tab
    a. click temperature tab
    b. right click on graph area and select ‘save data to .cvs file’
12. Open file in excel and analyze by temp and time that corresponds with the time of experimental data that was collected

Citations:

Virens, Joseph & Cree, Alison. (2018). Further miniaturisation of the Thermochron iButton to create a thermal bio-logger weighing 0.3 g. *Journal of Experimental Biology.* 221, 1-6.

Gur, Mutlu Kart & Gur, Hakan. (2015). Age and sex differences in hibernation patterns in free-living Anatolian ground squirrels. *Mammalian Biology*. 80, 265-272.

Testing the TOBEC equipment *Could not finish - machine missing parts*

Tess Laub, Lauren, Sarah Coose, Caitlin, Skylar

What is a Tobec?

- Tobec: Total body electrical conductivity
- Measures total body electrical conductivity to estimate lean body mass
- Based on the principle that organisms placed in an electro-magnetic field perturb the field; fat free mass has a higher water and electrolyte content than fat mass; strength of field depends on electrolytes found in an organism's body water
- Passing through the electromagnetic field of the coil absorbs heat energy
- The loss of energy detected is an index of the conductive mass of the body
- Can be affected by hydration levels, temperature
- Egg measurement: total body electrical conductivity - I = k*(E-B)/R
- B = reading within chamber, E = empty chamber, R = reference number, k normalization index
- Total lean mass (total fresh egg mass - lipid mass)
- Chilling eggs to 0 degree celsius

What does Tortec do?

- Estimate lean body mass
- Test electrolyte content in body by encasing subject in electromagnetic field-generating cylinder

Applications

- Used mostly with birds
- Used with various eggs
- Used to measure fat-free body mass of diabetic rats treated with an insulin-like drug (Uddandrao, 2019)
- Originally designed to estimate fat content in refrigerated hamburger meat (Wunder, 2000)
- Used to estimate lipid mass - noninvasive compared to lipid extraction (Pearce, 2008)

Usefulness of a Tobec

- Entirely non-invasive
- Fast measurement (few seconds)
- Relatively high accuracy due to ease of quick repetition

Limitations

- It can be affected by hydration status
- High cost
- Not widely available
- Based on regression equations

Instructions (How does a Tobec work?)

To perform a phantom scan:

1. Place the proper phantom for the detection chamber that is being used near the system.
2. Begin at PRESS BUTTON TO BEGIN SCAN. As indicated, press the STEP button to initiate the SCAN procedure.
3. When SCAN appears, insert the reference phantom into the detection chamber. With reference phantom centered in the detection chamber, wait for the readings to stabilize, and then press the STEP button. When REMOVE SUBJECT appears, immediately remove the phantom and press the STEP button again. At this point, the CONTROL MODULE screen will display PLEASE WAIT for several seconds while the system is calculating the final result.
4. EVALUE = X. The value of the phantom should be within the specified tolerance limits stated on the phantom. Record the readings remove the reference phantom and press the STEP button. It is recommended that three acceptable phantom scans are completed before scanning your subjects.

Scan Procedure:

1. Start the scan procedure with an empty detection chamber.
2. Click on the background button to take subject readings which will be updated once per second. The background field will turn green while the SA-3000 is in background mode.
3. Wait for a stable background, defined as less than one count drift per second on average.
4. Click on the Subject button to take subject readings. The subject field will turn green while the SA-3000 is in the subject mode.
5. Insert the subject into the detection chamber.

6. Wait for a stable subject reading. It will take at least 5 seconds for the subject reading to stabilize
7. Click on the Background button to put the SA-3000 back into background mode. This will place the subject reading into the Scan results box.
8. Remove the subject from the detection chamber.
9. To scan the same subject again, repeat the procedure starting at step #3. The SA-3000 will be in background made after completing step 7.
10. To save the average scan result, click on the E value button to place the normalized E value in the E value column of the current row of the worksheet.