Lab 3 – Diet and Digestion

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## Objective

The purpose of this study is to:

1. Understand the **relevancy** of diet quality to understand the physiology of animals
2. Use **scientific practices** to quantify and compare diet diversity and digestibility
3. Understand how the environment and animal morphology influences the physiological process of digestion
4. Archive digital data on diet diversity and digestion by your animal for future **iteration, collaboration** and **discovery**

**Preparation**

1. Obtain foil packets of crop contents (separated by species-parts), gizzard contents, and fecal pellets that have been dried.
2. Be sure to wear proper personal protective equipment—safety glasses and gloves (lab coat is less necessary for this lab).
3. Find the following hard copy and electronic (digital) data worksheets
   1. Hard copy and digital “Master morphometrics” data sheet from previous lab. Make sure it is updated.
   2. Digital copy of “fecal pellet data” worksheet from previous lab
   3. Digital copy of “crop biomass” worksheet from previous lab
   4. Digital copy of “gizzard biomass” worksheet from previous lab



Example of long skinny shoots.

* 1. **New “master diet diversity function” worksheet**
  2. **New “crop diversity index” worksheet**
  3. **New “bite diameter” worksheet**
  4. **New “particle size digestion” worksheet**

1. Each pair should obtain your tools for conducting scientific practices:
   1. forceps (1-2)



Example of thick short shoots.

* 1. calipers (1),
  2. Sharpie (1),
  3. 10cm piece of masking/lab tape (two 4-6” strips to hang biohazard bag),
  4. weigh boats (15 small size, 4 larger size),
  5. set of 6 sieves (1 set per group of 4-5 people)
  6. Small plastic bags for keeping contents separate



Example of tiny Salix.

* 1. weighing scale (at 1.0 mg (0.001g) capacity, shared),
  2. 10% bleach (1 spray bottle for clean up),
  3. 70% ethanol (1 spray bottle for clean up),
  4. stack of paper towels.

1. Before you begin, **identify the groups who have woody plants from their crop contents**. Use “CropSpeciesPartsPtarmigan.ppt” to help identify the following to measure bite diameters described later:
   1. Long skinny shoots (may have buds, Fig 1)
   2. Thick short shoots (may have buds)
   3. Tiny salix (S. herbacea) twigs with buds

**Part 1. Obtain Dry Weights of Contents of Crop, Gizzard and Large Intestine**

1. Organize your foil packets with gut contents that were dried in order of
   1. Crop: Three woody plants first (long shoots, short shoots, tiny salix), then most abundant species-part to least
   2. Gizzard contents
   3. Fecal
2. If you have not already separated your crop contents into separate species-parts, do this NOW.
3. For each species-part from the dried crop contents: Measure weight of content + foil in “crop biomass” worksheet for each species-part you have.
4. For dried gizzard contents: Measure weight of content + foil in “gizzard biomass” worksheet
5. For dried large intestine contents (feces): Measure weight of content + foil in “fecal pellet data” worksheet
6. If you did not get a weight of your foil before drying, you will need to transfer each dried species-part, or gizzard, or fecal material into a tared weigh boat to get the dry weight.
7. Calculate (or directly measure) dry weight of content (weight of content + foil minus weight of foil recorded in previous lab) in each respective worksheet: “crop biomass” for species-parts of crop contents, “gizzard biomass” for gizzard contents, “fecal pellet” for feces.
8. Transfer contents (kept in foil is fine) into small labeled (BirdID, content type, group ID) bags for storage.
9. Calculate ratio dry weight of each sample in each respective worksheet by the equation (dry weight/wet weight).

**Part 2. Calculate Diet Diversity from Crop Contents**

1. After entering dry mass (g) for each species-part from crop, calculate diversity using equations in the spreadsheet “crop diversity index”: **Q2: What does diet diversity tell you? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**
   1. *See equation file in lab 3 content on Blackboard if you are curious as to how a Shannon diversity index is calculated*
2. Enter final diversity values from dry mass data in “Master diet diversity function” worksheet

**Part 3. Bite Diameter**

1. Select the most abundant woody species from your crop contents and open foil. If you do not have a woody crop species, find another group who does and help them measure their woody species
2. Open foil for your gizzard contents
3. Open “bite diameter” worksheet
4. Use calipers to measure diameter (mm) of at least 3 and a maximum of 5 woody plant parts of at least one woody species from your crop contents
5. Use calipers to measure diameter (mm) of 5 woody plant parts from your gizzard food content.
6. Calculate average diameter of each woody plant part from crop and gizzard and enter the average value in in “diet diversity and function” worksheet.

**Part 4. Particle Size as Indicator of Digestion and Preparing for Coumarins**

1. Measure and record the weight (without lid) of each sieve size (#1 is largest mesh, bottom is smallest mesh) so you can subtract it from contents of sieve later)
2. Arrange the sieves so the largest mesh is on top (#1), and progressively smaller mesh one down through the stack until the closed bottom container (#5) completes the assembly
3. Select your **most abundant crop species-part**
   1. Record the weight of a labeled 2.0mL microcentrifuge tube
   2. Transfer approximately 100 mg of dry biomass of your most abundant crop species-part into the labeled microtube and record the biomass on the tube.
   3. Tube should have the following written on the side or with tape: (Bird ID, crop, biomass of content in g)
   4. Place this labeled microtube aside. **This will be used for coumarin extraction in a future lab.**
4. With the remaining sample, complete the following:
   1. Make sure the dry sample is not clumped. Be careful not to break or damage particles, but you need to separate existing particles.
   2. Place the dry sample in the top container,
   3. cover it,
   4. gently shake it back and forth with horizontal motion.
   5. Remove the cover.
   6. Weigh sieve + contents for each sieve size. Or transfer from sieve to another tared container – whatever works best for you.
   7. Calculate dry weight of contents retained in each sieve mesh by (weight of sieve + contents) minus weight of sieve. Record weights in “particle size digestion” worksheet
   8. Calculate the % of total dry weight biomass for each sieve size and record in “particle size digestion” worksheet:
      1. mass of dry content in sieve #1/total dry mass of species-part)\*100
      2. mass of dry content in sieve #2/total dry mass of species-part)\*100
      3. repeat for all sieves where you have biomass
   9. Transfer contents from each sieve size into a labeled (Bird ID, content type, sieve size, group ID) and place all particle size bags into a single labeled bag for that content type.
   10. Store this bag with each particle size bags within it with the rest of your separated crop species-parts as they may be used for future labs.
   11. Completely remove all contents of each sieve size, clean with water, spray with 70% ethanol and wipe clean with paper towels between each sample type. **Make sure sieves are dry between samples and at END of lab.**
   12. You should have the following
       1. one 2.0mL microtube with 100 mg of your sample for extraction of coumarins for Lab #4
       2. 5 sizes of particles from the remaining sample – each with a dry mass recorded. It is possible that you will NOT have contents in each sieve size. If you do not have particles in a sieve size, record a zero (0 dw g) in that cell.
5. For your dried gizzard contents.
   1. Record the weight of a labeled 2.0mL microcentrifuge tube
   2. Transfer approximately 100 mg of dry biomass of your dried gizzard content into the labeled microtube and record the biomass on the tube.
   3. Tube should have the following written on the side or with tape: (Bird ID, gizz, biomass of content in g)
   4. Place this labeled microtube aside. **This will be used for coumarin extraction in a future lab.**
   5. Repeat #4 a-l above
6. For dried fecal pellets
   1. Record the weight of a labeled 2.0mL microcentrifuge tube
   2. Transfer approximately 100 mg of dry biomass of your dried fecal material into the labeled microtube and record the biomass on the tube.
   3. Tube should have the following written on the side or with tape: (Bird ID, feces, biomass of content in g)
   4. Place this labeled microtube aside. **This will be used for coumarin extraction in a future lab.**
   5. Repeat #4 a-l above

**Q3: What is the predicted relationship between bite diameter, beak morphology, grit morphology and particle size? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Q4: What observations about particle size would indicate higher digestibility of food? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Part 5. Short Report and Discussion**

1. **Starting at 3:15.** Clean up lab.
2. **Starting at 3:30**: Calculate diversity, average bite diameter, %mass of particle size for crop, gizzard and fecal material in class “diet diversity and function” worksheet:
   1. Select at least one hypothesis to test with data.
   2. Ask for feedback on hypothesis.
3. **Starting 4:00**. Discuss questions, finalize independent projects related to diet diversity and record KSAs.

**What you should have prepared at end of lab**

|  |
| --- |
| 1. Dried samples in bags separated by: intestinal segment (crop, gizzard, large intestine) and separated within these by content type (species-part in crop, gizzard content, feces) and each content type (most abundant species-part from crop, gizzard content, feces) separated by particle sizes. **Storage: all dried contents types in a ziplock at room temperature.** |
| 2. Three 2.0mL microtubes labeled with Bird ID, sample type, g dry mass. These should each contain 100 mg dry weight of the following: 1. Most abundant species-part from crop; 2. Gizzard content; 3. Fecal material. **Storage: place in vial holder with animal ID and stored in lab at room temperature to be used for extracting coumarins in next lab.** |
| 3. Data on 1) diversity of crop content; 2) average bite diameter for largest woody plant in crop and in gizzard; 3) dried biomass of each sieve particle size for most abundant crop species-part, gizzard content, and feces shared in class spreadsheet for your bird. **Storage: in “Master diet diversity and function” worksheet on google drive. Brecken will transfer to Blackboard** |
| 4. Draft short report related to diet quality. **Storage: Submitted first independent short report the following week.** |
| 5. Final idea approved by instructors for independent projects related to morphology and digestion. |

**Discussion:**

1. **What are the expected potential relationships between particle size and the following –find a published paper that investigates these relationships:**
   1. Diet diversity or evenness
   2. Grit size
   3. Bite diameter of woody plant
   4. Feeding behavior
   5. Body size, sex or age
   6. Morphology of gizzard (muscle thickness, liner)
   7. Time of year or geographic location where collected

**2. What did you gain today**:

Knowledge:

Skills:

Abilities:

**3. Next week’s tasks**:

* Digestion short report due (30 min – 1 hr)
* Meet in lecture at 12:00 on Monday and Wednesday
* Meet in lab at 1:30 on Wednesday, close-toed shoes