Lab 2 – Avian Dissections Intestine Morphology and Diet Preparation

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Protocol revised by **Lauren Willows-Munro November 13,2019**

## Objective

The purpose of this study is to:

1. Understand the **relevancy** of game birds for: advancing science, management of natural resources by state agencies, understanding the value of hunters, connection with the land and public
2. Use **scientific practices** to dissect and measure the intestinal tissues and link these to interactions with the environment and physiological function of animals
3. Use **scientific practices** to measure features of intestinal contents and prepare contents for future analysis of dietary quality
4. Archive digital data on morphometrics of your animal to compare to past data and for future **iteration, collaboration** and **discovery**

**Preparation**

1. Set the intestinal contents (crop, gizzard, small intestine, ceca, large intestine) out to thaw overnight in a bin within the fridge (-4°C).
2. Be sure to wear proper personal protective equipment—safety glasses, gloves, lab coat.
3. Obtain the same carcass and tissues you had in lab #1. You will need to separate the following for digital archival of morphometrics:
   1. the head (for photo of beak),
   2. both wings cut at wrist to include primary feathers for photo
   3. entire intestinal tract (crop, proventriculus, gizzard, small intestine, both ceca, large intestine to cloaca),
   4. Information notes and data sheet that is linked to your bird
4. Find the following data worksheets: **(needs to made or modified for future labs)** https://docs.google.com/spreadsheets/d/1vxeCpLkkfQGenDSKPdHN5KT6v7-Hyvyz7Ksf38-m0w4/edit?usp=sharing
   1. Hard copy and digital “Master morphometrics” data sheet from previous lab. Make sure it is updated.
   2. New “fecal pellet data 2019” worksheet
   3. New “crop count 2019” worksheet
   4. New “crop biomass 2019” worksheet
   5. New “gizzard 2019” worksheet
5. Each pair should obtain your tools for conducting scientific practices:
   1. dissection tray (1),
   2. forceps (1-2),
   3. scalpel blades and handles (1-2),
   4. dissection scissors (1),
   5. absorbent paper (1-2 layers),
   6. plastic bags: 1 gallon-size Ziploc bag (for carcass disposal), 3 quart-size Ziploc bags (for tissue collection), 10 coin-size Ziploc bags (for tissue collection),
   7. Sharpie (1),
   8. 10cm piece of masking/lab tape (two 4-6” strips to hang biohazard bag),
   9. weigh boats (15 small size, 4 larger size),
   10. metric ruler (2),
   11. measuring tape (1),
   12. calipers (1),
   13. aluminum foil (approximately ½ meter length),
   14. measurement tray with metric scales on it (for photo of morphometrics),
   15. Box for organizing foil packets to get dry weights of samples
   16. access to weighing scale (at 1.0 mg (0.001g) capacity, shared),
   17. Access to tripod and camera for photo shoot of morphology
   18. 10% bleach (1 spray bottle for clean up),
   19. 70% ethanol (1 spray bottle for clean up),
   20. stack of paper towels.

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| **General order of activities for recording data** |  |  |

**Part 1. Wet Weight of Contents of Crop, Gizzard and Large Intestine**

1. Write bird ID and weight of large weigh boat on weigh boat and transfer crop contents into the labeled large weigh boat.
2. Obtain and record wet weight of entire crop contents (without tissue) in “Master morphometrics” data sheet
3. Keep contents homogenized and spread out to fill bottom of weigh boat. Set the “crop boat” aside.

**Q1. What types of food do you observe? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

1. Observe the morphology of the crop tissue.

**Q2: What advantage and limitations does the crop have for animals? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_;**

**Q3: How might birds with different foraging behaviors differ in crop tissue and morphology? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

1. Carefully cut longitudinally along the gizzard to fillet it open.
2. Write bird ID and weight of large weigh boat on weigh boat and transfer gizzard contents into the labeled large weigh boat. Separate gizzard contents using (gloved) fingers.
3. Obtain and record wet weight of entire gizzard contents (without tissue) in “Master morphometrics” data sheet
4. Keep contents homogenized and spread out to fill bottom of weigh boat. Set the “gizzard boat” aside.

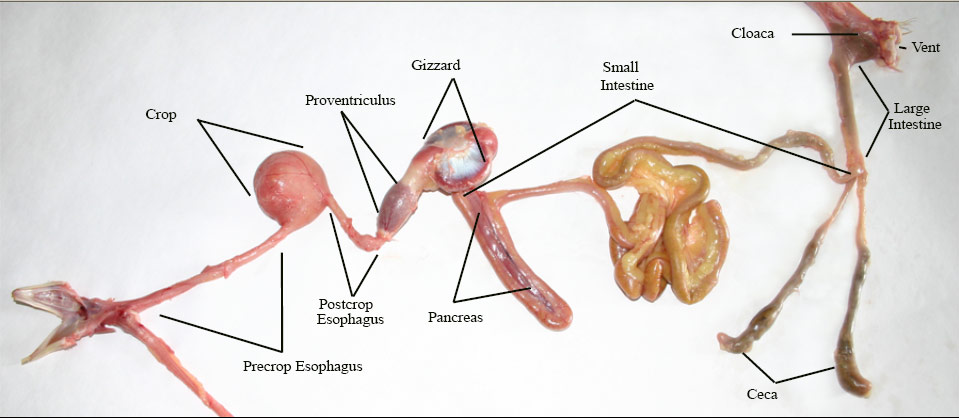
**Q4. How does the morphology of food and contents compare to crop contents? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

1. Observe the morphology of the internal gizzard liner and gizzard muscle.

**Q5: What advantage and limitations does the gizzard have for animals? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_;**

**Q6: How might birds with different foraging behaviors differ in gizzard tissue and morphology? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

**Part 2. Lengths of Compartments of Intestinal Tract**

1. ***Carefully*** remove the mesentery (connective tissue) between each intestinal segment and spread the entire tract out. **You should be able to identify the crop, proventriculus, gizzard, small intestine, both ceca, large intestine to cloaca**. Torn intestinal tracts smell really bad…
2. As you unfold the proximal end of the small intestine, remove the pancreas. Record mass of pancreas in wet weight (ww) and place it in labeled small plastic bag.
3. Cut the proventriculus from as close to the gizzard as possible. Record mass in ww in “Master morphometrics” data sheet
4. Measure length of each intestinal tract segment with a tape measure and record in “Master morphometrics” data sheet. Each segment should be straight and laid down gently **without stretching them** 
   1. small intestine from gizzard to junction of caeca;
   2. each cecum from junction with small intestine to tip; and
   3. large intestine from caeca junction to lip of vent including cloaca.
5. Calculate the length of full intestines from small intestine (just distal to gizzard) to cloaca (sum of a, b, c above).

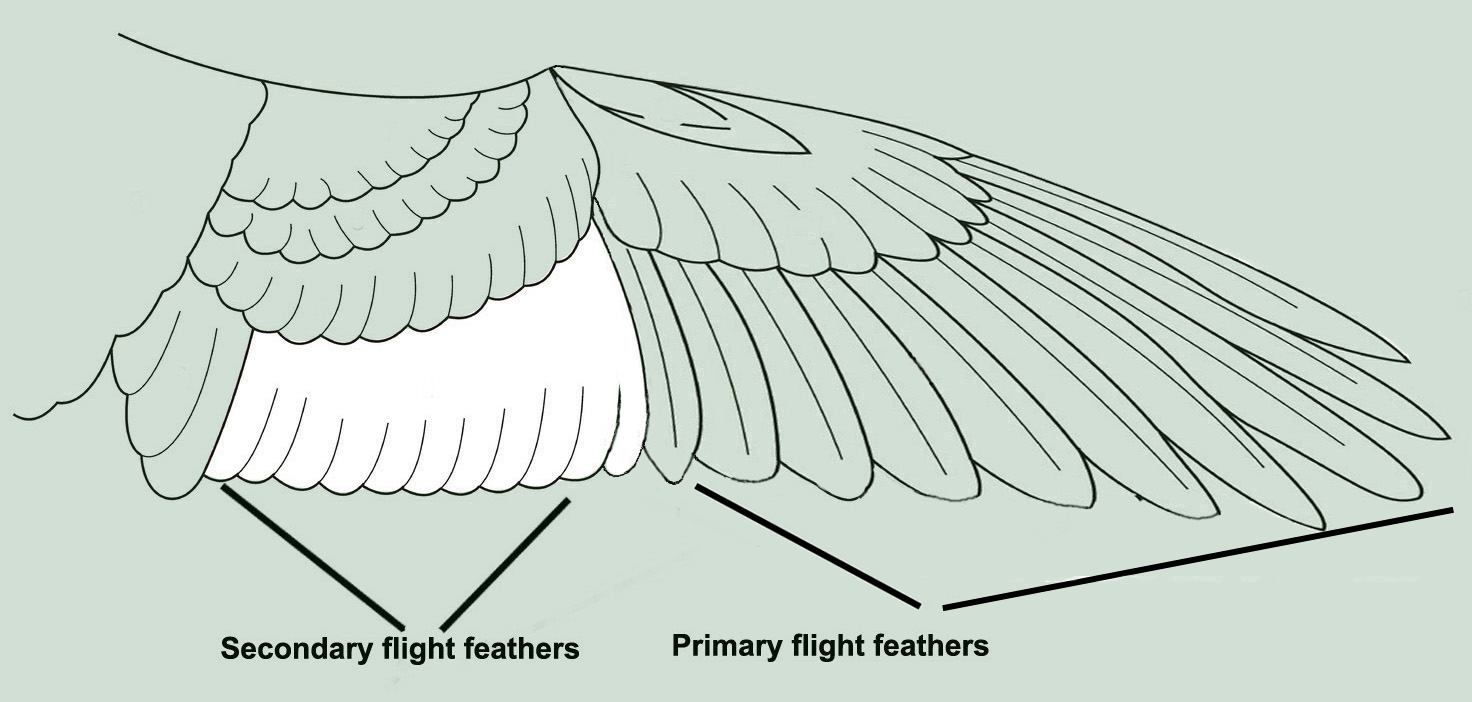
**Part 3. Size of Fecal Pellets and Preparation**

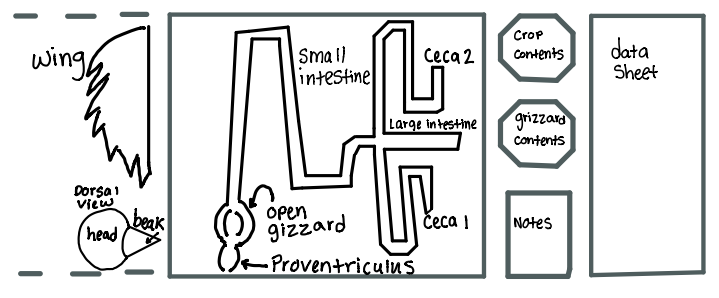
1. Squeeze out contents of large intestine without damaging pellet shape.
2. Record wet weight of entire large intestine contents (all fecal pellets) in “fecal pellet data” worksheet
   1. Cut foil, label with: bird ID, fecal, weight of foil.
   2. Enter weight of foil in “fecal pellet data” worksheet
   3. Add wet contents of fecal pellets to foil and record weight of fecal + foil in “fecal pellet data” worksheet.
   4. Calculate wet weight of fecal pellets (weight of foil minus weight of wet fecal pellets + foil) in “fecal pellet data” worksheet.
3. Use calipers to measure width and length (mm) of at least one and maximum of 3 intact fecal pellet and enter data in “fecal pellet data” worksheet
4. Calculate volume of measured fecal pellets as π x r2 x h, where π = 3.14, r = ½ of your calculated width in mm, h = your calculated length in mm.
5. Calculate average width, average length and average volume of fecal pellets for your bird

**Q7: What correlations might we make with fecal pellet size and why might this be useful to know? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

1. After getting size of fecal pellets, fold labeled foil so you can see bird ID and fecal and place foil packet in box for your group for transfer by instructors to drying oven. This foil packet will be used next week for:
   1. Determining dry weight of fecal pellet
   2. Calculating fraction dry weight of fecal pellet
   3. Determining particle size to predict digestibility of food
   4. Extracting and comparing relative quality of UV fluorescent secondary metabolites from feces
2. Record if you see any internal parasites and collect in labeled (Bird ID, parasite) vial of appropriate size and add enough 70% ethanol to cover the parasite.

Wrist

**Part 4. Digital Archival and Analysis** (for consistency, iteration, and future discovery)

1. Cut off the head and place dorsal view (see top of head) so beak aligns with outer bottom left size of measurement tray
2. Cut each wing at the wrist and place one of them with #10 primary along top outer left side of measurement try. Before photo, make sure the primaries are spread out
3. Place each weigh boat with crop contents and gizzard contents on right side of measurement tray.
4. Lay out the “re-assembled” intestinal tract sections on the measurement tray starting with
   1. proventriculus (aligned with left bottom inside of measurement tray,
   2. then empty filleted-open gizzard (so muscle thickness can be seen),
   3. then small intestine to junction
   4. Arrange both ceca on either side of the large intestine.
   5. Each bend in intestinal segment should have same length.
   6. Intestines should be straight and laid down gently without stretching them or allowing folds.
5. Add any notes from bird to the side of measurement tray (wherever there is room)
6. Add completed data sheet from your bird that MUST include your bird ID, body mass (indicating if this includes pectoral muscle or not), sex, and age in addition to lengths of measurements that can also be determined from digital photo.

**Q8: Why and how might in class measurements differ from those determined digitally? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

1. Confirm the following before and after the photo
   1. Crop and gizzard contents are homogenized in their separate weight boats
   2. All data is entered in hard copy of data sheet and visible in field of view
   3. Notes and data are in focus in photo
   4. Scale of metric units on measurement tray is visible in photo
   5. Primaries of feathers are separated and #10 primary is lined up with metric units (you might have to hold them in place for photo)
2. Take a picture of your beak, wing, measurement tray, contents of crop and gizzard and data for archival
3. After confirm above - enter picture ID to the “Master morphometrics” data sheet.
4. Place empty crop tissue, empty gizzard, and remaining part of intestinal track in their own labeled (Bird ID, tissue type) plastic bag.
5. Use digital image to calculate the length of the small intestine for your bird and enter value in the “Master morphometrics” data sheet.

**Part 5. Crop Content Separation and Preparation**

1. Open New “crop count” worksheet
2. After photo of crop content is confirmed, separate each species based on morphology into their own small weigh boat
   1. confirm the plant species-part is in the crop worksheets.
   2. Write plant species-part on each separate weight boat
   3. Compare your plant species-part with other groups. If you differ in identification of plant species-part with another group tell instructors so we can confirm this information before moving forward.

**Q9: How do your species-part differ from other groups and why might this occur? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

1. Record the count per species-part in “crop count” worksheet.

**Q10: Why is the number of plant species-part important, what might it predict? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

1. Record wet weight per species-part in “crop biomass” worksheet
   1. Cut foil, label with: bird ID, plant species-part, weight of foil. Enter weight of foil in “crop biomass” worksheet
   2. Add wet contents of each species-part to foil and record weight of species-part + foil in “crop biomass” worksheet.
   3. Calculate wet weight of each species-part (weight of foil minus weight of species-part + foil) in “crop biomass” worksheet.

**Q11: Why is the wet mass of plant species-part important, what might it predict? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

1. Fold labeled foil so you can see bird ID and plant species-part and place each foil packet in weigh boat for your group for transfer by instructors to drying oven. These foil packets will be used next week for:
   1. Determining dry weight of plant species-part
   2. Calculating fraction dry weight of each plant species-part
   3. Calculating diet diversity based on dry weight
   4. Measuring bite diameter as a proxy for beak morphology and diet quality
   5. Determining particle size to predict digestibility of each plant species-part
   6. Extracting and comparing relative quality of UV fluorescent secondary metabolites from each plant species-part

**Part 6. Gizzard Content Separation and Preparation**

1. Record wet weight of gizzard filtrate in “gizzard” worksheet
   1. Cut foil, label with: bird ID, filtrate, weight of foil. Enter weight of filtrate foil in “gizzard” worksheet
   2. Add wet contents of combined grit to foil and record weight of filtrate + foil in “gizzard” worksheet.
   3. Calculate wet weight of grit (weight of foil minus weight of filtrate + foil) in “gizzard” worksheet.
2. Fold labeled foil so you can see bird ID and filtrate and place each foil packet in weigh boat for your group for transfer by instructors to drying oven. These foil packets will be used next week for:
   1. Determining dry weight of gizzard filtrate
   2. Determining if grit morphology influences particle size of gizzard material to predict digestibility of diet
   3. Extracting and comparing relative quality of UV fluorescent secondary metabolites from gizzard contents

**Q12: What is the relationship between size or shape of grit, diet particle size and digestion of diet? \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_**

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

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| 1. Bird ID and your group name on outside of Gallon ziplock bag with the following inside in individual bags each with Bird ID and description of contents: bird carcass + 2 wings removed at wrist with Bird ID; organs in individual bags: liver, heart, empty crop, empty gizzard, small + ceca + large intestine. **Storage: -20C freezer.** |
| 2. Picture of morphology (head, wing, guts), notes and completed morphology data sheet. **Storage: on camera and transferred to google drive (and on your individual phones).** |
| 3. Foil packets containing: individual species-parts from crops, gizzard contents, feces from large intestine. To be dried by instructors and used next week to measure dry weights, particle size, and to prepare coumarin extracts. **Storage: in single container with animal ID to be transferred by Brecken to drying oven in Forbey lab.** |
| 4. 1 mL of DI water extract of gizzard contents in 2mL microtube. To be frozen by instructors and used in week 4 for coumarin assay. **Storage: place in vial holder with animal ID and placed in -20C freezer.** |

**Discussion:**

**1. What measurements could we make on game birds that would benefit management of natural resources by our state agencies**?

**2. What information from hunters might be useful to better understand morphology and diet of game birds**?