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**Protocol for northern bobwhite (*Colinus virginianus*) eggshell sample collection**

**Overview**

Research on northern bobwhite quail dates back to the 1920's with Herbert Stoddard, and yet chick survival and recruitment has been difficult to determine and is considered to be the least understood aspects of bobwhite ecology. Previous methods to estimate survival include: flush counts, mark-recapture with patagial tags, radio telemetry, and the use of thermal imagery (forward-looking infrared [FLIR]). These methods have produced various survival estimates that are likely much lower that actual rates due to incomplete counts, brood amalgamation, and tagging attachment effects on survival.

The objectives of this research are to test novel techniques to estimate northern bobwhite chick survival, and to understand the levels of accuracy, precision, and cost among the methods. We will use genetic markers on hatched eggshells and feathers from fall trapped individuals in a capture mark-recapture study.

**Supplies**

- *Coin envelopes*
- *Quart or gallon freezer ziplock*
- *Sharpie*
- *Gloves*

**Collection**

Nest Monitoring

Check nest status daily once incubation begins (incubating, recesses, terminated). When nests are approaching the expected hatch date, begin checking nests twice daily, once in the morning and once in the afternoon. When the bird is no longer in vicinity of the nests, walk in to determine the nest status (abandoned, hatched, failed). It is important that we get to the eggs as quickly as possible. DNA degrades rapidly in the heat and humidity, so the sooner we collect samples the better our chances of extracting quality material.

Determining Nest Fate

**Abandoned**

Eggs are whole and remain in or immediately adjacent to the nest bowl.

**Predated**

Most or all eggs have been consumed. Eggshell pieced may be scattered around the nest bowl.

**Hatched**

Eggs are in or adjacent to the nest bowl. Distinct pipping around the tops pf the eggs with egg tops still attached via the membrane or laying in nest bowl.

Collection

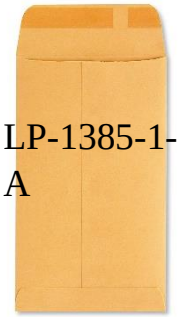
For the objectives of this project, we are only interested in collecting eggs from hatched nests. When collecting eggs, always use gloves (a single gloved hand if fine). We are after the inner membrane of the eggshell, and as such need to avoid contact with the inner part of the shell during collection. When collecting eggs try to grab them from the opposite end they pipped out from.

i. Note the number of hatched eggs.
ii. Each egg will get its own coin envelope.
iii. If the top is still attached, leave it so
iv. Tops can detach during the hatching process. Any lose tops can be placed together in a single envelope labeled "tops".

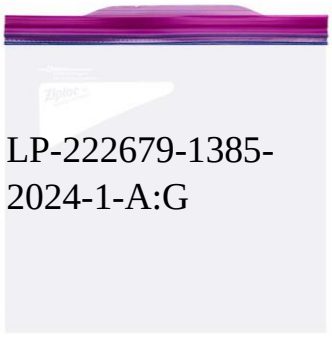
**Labeling and Storage**
For each samples we will need following information:

- Site samples came from (TT, LP, RRYP, or Escape)
- Bird/Band ID
- Frequency
- Year
- Nest attempt
- Sample ID (A:Z)**

**Note: each egg and envelope will have its own sample ID








Label each coin envelop with the with the following information:

Site – Frequency – Attempt – Sample (A:Z)

Place all coin envelopes into a single labeled ziplock with the following information:

Site – Bird ID – Frequency – Year – Attempt – Samples (A:Z)

Samples can then be stored in a freezer.

**Labeling Examples:**
LP-222679-1385-2024-1-A:G
This sample would indicate that this bird from Livingston Place, 222679_151.385, is its first clutch of 2024 and hatched 7 eggs in this clutch.

TT-242532-9487-2024-2-A:E
This sample would indicate that this bird from Tall Timbers, 242532_149.487, is on its second attempt and hatched 5 eggs in this clutch.

**Central Florida:**
For samples stored at room temperature, add desiccate packets into the ziplock bag containing each nests' samples. The desiccate will help remove moisture that can degrade the DNA.