Ketterson / Nolan Research Group Collection

This document is part of a collection that serves two purposes. First it is a public archive for data and documents resulting from evolutionary, ecological, and behavioral research conducted by the Ketterson-Nolan research group. The focus of the research is an abundant North American songbird, the dark-eyed junco, *Junco hyemalis*, and the primary sources of support have been the National Science Foundation and Indiana University. The research was conducted in collaboration with numerous colleagues and students, and the objective of this site is to preserve not only the published products of the research, but also to document the organization and people that led to the published findings. Second it is a repository for the works of Val Nolan Jr., who studied songbirds in addition to the junco: in particular the prairie warbler, *Dendroica discolor*. This site was originally compiled and organized by Eric Snajdr, Nicole Gerlach, and Ellen Ketterson.

Context Statement
This document was generated as part of a long-term biological research project on a songbird, the dark-eyed junco, conducted by the Ketterson/Nolan research group at Indiana University. For more information, please see IUScholarWorks (https://scholarworks.iu.edu/dspace/handle/2022/7911).

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Note - there are some changes here from "So you found a nest...." this document should take precedence where there is a conflict.

A. Egg steroids. Goal: perfect clutches for steroids, 10 from T-males, 10 from C-males.

1. **Find nest during building**, hopefully before egg 1 has been laid. At least initially we will take clutches from nests found during laying, i.e., any clutches found before another egg appears. We will also take clutches that are parasitized by cowbirds, because we can use these for preliminary data to tell us whether this line of research is worth following. But the long-term goal is 10 and 10 perfect clutches in which we are certain of the order and there is no parasitism.

2. Identify the parents. Use tape (judiciously!). Try being there at the time the egg is laid (this time of year by 0645, earlier later on) because male may accompany female to nest and might be particularly responsive to a brief playback at that time. Other times will work as well because males stay with laying female while they are foraging. Go to territory where nest is and listen for call notes. The male will sing occasionally and accompany the female nearly all the time. When you return each day during laying to mark eggs (see below), use each opportunity to determine adults. If you find out, be sure to report IDs right away (!!!!), so others don't spend time trying to do something you have already done. If you have not IDd the male but know who the female is (or vice-versa), then at least this year, I think we should take the clutch anyway. BUT, then it must be the highest priority in the next 72 hours to see who the female's mate is. If we don't know who either bird is, then I do not think we should take the clutch.

3. **Mark the eggs each day.** The rationale for the project depends on knowing each egg's order in the laying sequence, so this is critical. Until further notice, please mark with a soft pencil. Carefully remove egg from nest, holding it gently in your left hand. Use side of lead on pencil to mark with a I, II, III, or X. Don't poke the shell! When returning egg to nest take care. Nestle egg in your fingers and let your fingers lead the way into the nest to avoid puncture of the egg by vegetation or nest material. Release egg gently into cup of nest. Beware, sorting this out later will depend on writing the correct dates in the nest logs. Check the calendar each time you record the date regarding the presence of 2 eggs, 3 eggs, etc.

4. **Remove the clutch** and transport it safely back to the lab on the first day on which no new egg appears (i.e., the day after the last egg is laid). Clutches will often be 4 eggs in the early season, 3 eggs later on. That means you get the clutch early in the morning on the day after laying is complete. Every hour of exposure is time when predators may take the whole clutch and all is lost, and once incubation begins the steroids will begin to be used by the developing embryo. Place eggs in well marked container stuffed with cotton. One of the microhematocrit tube holders will be good for two eggs.

5. **Measure and weigh the eggs** after returning to the lab. Each egg should be measured, long axis and wide axis, and weighed to the nearest 0.1 g on a top-loading precision balance. Take care, if the egg breaks the sequence is no longer perfect. However if the yolk is intact it can be used, so do not discard even if egg is cracked.

6. After measurements are complete, place egg in a snap cap vial marked with NestID and egg number using time tape and permanent sharpie. Also write key info on the cap of the vial. Store the vials in the freezer in a well labelled container that will prevent egg smashing.
6. Carefully fill out data sheets with weights and dimensions as well as nest ID, parents' IDs and the usual (location, date, etc.)

**B. Sex ratio.** Goal: Determine sex ratio of broods as close to hatching as possible. If we assume that 50% of the nestlings produced by T- and C-males are male, then demographic data show that a higher percentage of males return than females, particularly from the nests of T-males. But we need to know when this bias in sex ratio appears - at laying, by hatching, during the first winter, etc. Said another way, we have many, many blood samples from nestlings at day 6, and these can be used to determine the sex ratio at that time. But there can be mortality between hatching and day 6 that could alter the sex ratio, and we have no information on this point.

1. After hatching is complete (the day after the last young has hatched), bring any unhatched eggs back to the lab. Make a determination as to whether the egg developed by external examination and then freeze the egg just as you would with the eggs we bring back at the end of laying (see above). Be sure to note on nest logs and on data sheet for unhatched eggs. If the egg developed, we may be able to determine its sex later from embryonic tissue.

2. The aim is to get blood samples of less than one microhematocrit tube (10 ul will do it, see e-mail from Alex Buerkle) from very young nestlings - on day 0 if possible, or day 1 if not, or day 2 if not that. Freshly hatched nestlings only weigh 2.5 g and seem very fragile. Still I am optimistic. The carotid artery is highly visible, and because nestling blood is thin, we may be able to get samples on day 0. We will isolate the DNA, amplify the product using PCR, and identify sex-specific sequences on the female chromosome. If the sample really is just 10 ul, then it would be good to store it in 100ul of Longmire's in 500ul eppendorf tubes. But if the volumes are quite variable, it will do no harm to put a small volume of blood in the usual Volume of Longmire's.

Note, it is extremely important that the Longmires and the eppendorf tubes NOT BE CONTAMINATED with human sequences. Use gloves when aliquoting and don't let foreign matter get into the eppendorf tubes. Also be sure to get all the DNA you can from the microhematocrit tube.

This will require careful monitoring of hatching. Also we will need to mark the nestlings with nail polish or magic marker so we can keep track of who's who until they reach banding age. That way if one disappears, we will know which one it was!! Nail polish works fine, but it can rub off, so it needs to be applied very carefully (a piece of grass dipped in the bottle works better than the brush to mark just the toes and not the foot - it takes a sec to dry, so think of this as an important task that needs to be done carefully). Since we need to return to the nests to renew the polish on day 3, we will take all measurements this year at day 0, day 3, day 6, and fledging (tarsus, mass, condition). If nestling weight sheets need to be modified, please anticipate and do this now.

**C. Female condition and physiology**

Goal: Capture 5 females mated to T- and to C-males while they are laying and bleed them to determine hormonal state as well as condition while not causing them to desert.

Basis for question is that if females differ in the levels of steroids they deposit in eggs or in the sex ratio of the young they produce, they must also differ in their own physiology. The key hormones may be cort as well as estradiol and T, so it would be best to reduce stress during capture. The idea would be to establish bait piles in the vicinity of the nest, but NOT so close as to attract chipmunks. Certainly if there are established bait areas nearby use those. Traps are probably better than nets.
You could have several traps going simultaneously, just be sure to record everything about the amount of time involved (time trap opened, time last checked, time as you approach trap, begin to bleed, etc. A sample of 100ul of plasma would be ideal. Don't harm the female!

D. Nestling development - more later

See above, we want to know mass, condition, and tarsus on day 0, day 3, day 6, and at fledging.