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).

April 18, 1995: REVISED VERSION OF MAY 29, 1992, REVISED AGAIN MAY 26, 1993, AFTER COMMENTS BY VN, LC, MHC, AND ZIG:

SO YOU FOUND A NEST.....

Congratulations! Each nest is special and so are the people who find them. [Some of the old-timers may not think that this is a big deal, but REAL students of juncos never tire of finding nests and never fail to appreciate the uniqueness and importance of each one.]

Here's what to do after you find a nest and while you are still in the field: **Note the stage of the nest and decide whether you need to do something right away. Identify the adults. Mark the location.**

1. **Nest stage**

Is the nest being built? Does it have eggs or young? How many eggs or young? Write careful notes in the field so you do not have to rely on your memory.

Building stage:

If the nest is being built, note its stage: rootlets, moss, grass, deer hair, etc.? Then **back off slowly** and watch for awhile. It is very important that you (1) do not frighten the female away and (2) do find out who the adults are by identifying the bands.

Empty nest:

If the nest is complete but empty, there is always the chance that it was built last year or built this year but has already failed. A good test to determine whether a nest is active is to place a tiny leaf in it. If active, the female should have removed it by the next time you visit the nest (make a note to yourself that says you put in leaf; later transcribe it to the nestlog).

Nest with eggs:

If the nest has eggs, note (1) how many, (2) whether they were laid by juncos or cowbirds, and (3) whether they are developed.

If there are fewer than 3 or 4 eggs, then the female may still be laying. Note whether the eggs are warm by touching them.

It is possible to tell very fresh eggs (which transmit light and are *translucent*) from eggs in which the embryos have detectably begun to develop which are *opaque*. This occurs about 3-4 days after incubation has begun. Just before hatching, eggs will begin to look '*chalky*.' The day before hatching, the eggs will pip, which means the soon-to-be nestling has made a rough spot in the shell while attempting to break free. Such rough spots are detectable with fingertips (or lips).

To determine stage of development, you can hold an egg up to sunlight or shine a flashlight through it. **However, be careful when handling eggs**. You don't want to crack or drop one. Except for 'fresh' and 'chalky and pipped,' it is virtually impossible to age eggs in the field and therefore to predict when they will hatch. You just have to keep going back to check.

Nest with young:

If the nest has young, you need first to count how many and decide whether they are juncos or cowbirds. Juncos are smaller than cowbirds and have gray down rather than white.

To decide whether to weigh and measure the young you must first decide how old you think they are. To prepare for this read the descriptions in the book entitled *Instructions*, look at the pictures in that notebook, and look at the graphs of body mass and tarsus length in the reprint that is attached to the bulletin board in the lab.

If the young are less than day 6 (hatching day = day 0), you should weigh and measure them in order to estimate their age from their stage of development. If they are day 6 (or 7), you should band and bleed them. If they are day 8 or older, **you don't want to touch them** or you might cause them to fledge prematurely (i.e., prior to day 12 early in the season, day 11 later on). Young that leave the nest early have a chance of surviving, but it is lower than if they stay in the nest for the full time.

If they **are** day 12 and ready to leave the nest, you may have to grab them now and band and bleed them or you **won't get another chance**. However think carefully before you do this.

As a guide, on day 6, the birds have long sheathed feathers on their wings, but the barbs have barely begun to break through. On day 7, they will look like little brushes and it is still okay to bleed them. By day 8 their bodies appear covered with feathers and you don't want to disturb them. They look quite similar between day 8 and 11 (most of the change is going on where you cannot see it, underneath the bird.) If they look to be fledging age (e.g., bright eyed and fidgety with short tails that look to be 15 mm long or longer), then you can grab them, **but do it all in one motion or they will run past you**. Again, you get only one chance.

Family of newly fledged young:

Every once in awhile you will come upon a family of newly fledged, *unbanded* fledglings (adults calling loudly, instead of finding a nest you flush a fledgling that can barely fly). Now this is a challenge. If they are young enough to run down, then catch them and band and bleed them. Even if you are able to catch only one, it will allow us to get a DNA sample and follow the family. Use your ears to try and determine how many young there are.

If a young has left the nest today or yesterday, it will fly weakly and you may be able to grab it before it flies at all: move slowly and quietly, extend your hand until the bird is within reach a foot or so away, then strike like a snake and grab it. If it flies, try to run it down (it will weaken with each flight), Also try to direct it toward a goal where you will have an easier time getting at it. If it ascends out of your reach, you may be able to shake the tree, hit the branch

with a stick, etc. Again, try to make it fly in a direction favorable to your catching it. Repeat with other siblings.

If the young are strong fliers it means they have been out of the next for several days. In that case you will not be able to run them down.

2. **Identify the adults**

Every time you find a nest (or visit one), try very hard to identify the adults! The first thing we will have to determine when you get back to the lab is whether the adults have been bled and whether the male has been implanted, so you have to know who they are. Decide whether the adults you see are the "parents." Did either one carry, nesting material (or food for nestlings). Did you see them deliver food or act defensively? Write it down (please). We need to know precisely how sure we are about the identify of the birds that 'think' they are the parents of the young.

3. **Mark the nest location**

Mark location with a flag on a nearby landmark. Put a very tiny piece of flagging very near to the nest. Write a nest number on the flag with a magic marker. If you are not sure of the number, then be sure to write on the flag when you next go back to the nest (hard to state strongly enough how important this is). Articulate to yourself a description of how to get there. If it's a hard one to find, make a trail of flags from a place where people will see it from the road or trail.

Be careful around nest sites so that we don't attract predators. Restore the vegetation, try not to trample it, etc.

Here's what to do after you return to the lab:

1. Make a nest log sheet, indicating what you found, how certain you are, and the reasons underlying your level of certainty. Write a really good description of how to find the nest on the nest log. This is very important. Please fill in all the blanks on the nest log sheets. There are some little yellow post-its on the bulletin board to help you estimate hatching dates, fledging dates, etc. by backdating.

2. Enter the nest on the blackboard so we know when to check status, when to expect hatching, weighing, etc. That is, enter the nest log number on the calendar with dates to weigh, bleed, fledge, etc. Write the nest ID under the lists of nests with eggs, unknown hatch dates, etc. People rely on this information to make up the daily lists, so it's important to remember to do this. Ask questions if you are not sure what to do.

Especially important:: if you visit a nest and find that it has failed, be sure to remove the nest ID from the calendar and the side lists on the blackboard.

3. Determine whether the adults have been bled and whether the male has been

implanted. If one of the adults is unbanded (and therefore unbled), put a post-it on the nest log and add it to the list of adults that need to be caught, banded, and bled. If it's been banded but not bled, add it to the list of birds that need to be bled.

ONCE A NEST HAS BEEN FOUND, HERE'S THE INFORMATION WE NEED TO GET FROM EACH ONE:

1. **Determining hatching date**

For nests found during building and laying, we need to return daily to determine how many eggs are laid. Incubation ordinarily lasts 12 days, and the female ordinarily begins to incubate the night before the day the last egg is laid (sometimes sooner as the season progresses). So if egg four is laid on June 1st, we expect hatching on June 12th.

If a nest is found after incubation has begun, please visit it at least every other day in order to determine when the eggs hatch.

Beware: we need to keep disturbance to a minimum. We don't want females to start flushing too readily because our visits have been too frequent. If they do flush easily they for fear become high risk candidates for predation. So please don't flush female unless it's necessary (e.g., you walked a mile to the nest and it's about to pour rain). Rather, if she's on, come back in ten minutes and see what's up. We should use the sign-up sheets to be sure that nests don't get visited too often. If someone else signed up to check the status of a nest, then leave it alone. If you agreed to check the status, be sure that you do it.

2. *Parasitism rates*

We want to know the rate of parasitism and how it varies annually and seasonally. We also don't want to have cowbird hatchlings in our nests. If a nest found during laying has a cowbird egg in it, leave it until at least two days after the clutch is complete. If you were to remove it earlier, you might cause the female to desert. When the clutch is complete (3 or 4 or 5 eggs plus cowbirds), then remove cowbird eggs. If there is more than one, and it is not too inconvenient, Lori recommends removing them on successive days, also to decrease the risk or desertion. Make careful notes and be SURE to record in the nestlogs what you did.

3. **Determining nest fates**

If a nest has failed, please note the circumstances. Was the nest lining torn out? Any feces in the nest? Any feathers from the female? Any bits of shell, partly eaten babies or color bands? Look around to see what you can see. Look beyond the nest itself, under it if it is elevated. Keep thorough notes.

We would like to know more about who the predators are. If someone were interested in taking charge of the mechanics, we might put flour near the nests (for footprints) or place a post with sticky tape near the nest (to see what mammals pass by and leave their hairs behind). Even cameras are a possibility, although we have not done enough research on this.

3. **Weighing and measuring young**

Traditionally, we have weighed the nestlings and measured their tarsi on DAYS 0, 3, 6

and also on fledging day. We began to skip the day 3 weighings last year. As of now, I would like to continue monitoring the number of eggs, the number of young at hatching, and the number of young at day 3, day 6, and fledging day. I am gong to postpone the decision as to whether to weigh young and measure tarsus length this year.

a. *Hatching day*

On hatching day (day 0), we need to weigh the young and measure their tarsi. Weighings and measurements are done after noon. Frequently, three eggs hatch one day, and one hatches on the next day. If any eggs remain unhatched, then please mark the *young* that have hatched and return the next day to process the young that have hatched in the meantime. Marking will let you tell them apart; nail polish applied lightly will last long enough.

If there are eggs that have still not hatched 24 hours after the first egg hatches, and it is obvious that they will never hatch, bring them back to the lab. Eggs that were punctured and are light in weight will never hatch. The same is true of eggs that are out of balance because they have dried out (such eggs roll in circles on a flat surface).

If there is still a chance they will hatch, then go back the next day (now day 2 for the earliest hatched young) and either process the last hatched young or bring the eggs back to the lab for processing (to determine whether the embryos developed so we can assess infertility and to preserve embryos for DNA). Note the return on any eggs to the lab on the nest-logs. We need someone to take responsibility for opening these eggs and deciding whether they show any signs of development.

Enter on nest log that young have been weighed. Fill out a nestling weight sheet. Nests should be aged according to the age of the majority of nestlings in the nest. If one hatches on Tuesday and three hatch on Wednesday, then Wednesday is day 0. If two hatch one day and two hatch the next, call it day 0 (for the nest) on the date that the first two hatched. Nests should be aged by their status in the P.M.

b. **Day 3**

On day 3, simply visit the nest and count the young. **Always** be on the look out for definitive information about who the parents are. Bands seen? food in bill? close approach and chipping?

c. **Day 6**

If by day 6, you are not sure who the parents are, ***watch the nest until both adults have fed the young and their bands have been identified.***

On day 6, band and bleed the young. Be sure to use unique band combinations (see Zig). Collect two or three tubes of blood from each nestling. Store the blood on ice (**don't let it freeze**!) and be sure not to mix up any samples. If you are not SURE whose blood is in a particular microhematocrit tube, please do not guess. It would be MUCH better to bleed the bird

again the next day.

Return to lab to process blood samples. AGAIN, BE SURE TO KEEP STRAIGHT WHO IS WHO! This is simply essential. If for **any** reason, you are not sure, please do not forge ahead. Write a LONG note about what went wrong and the basis for your best guess as to what is correct. As before, plan to bleed the nestlings again the next day or at fledging, if necessary.

d. **Between day 6 and fledging day**

Check the nest periodically between days 6 and 10 or 11 to determine whether it is still active (do parents chip?). Remember, do not touch the young or go too close to the nest after day 7 or you may cause premature fledging! Simply peek in. Don't harass the adults.

For nests that will permit long watches from a car, we may organize some daylong nest watches. We will want to structure these, so talk to Val or Zig or me about this. We would like to do six of these on nests from each treatment group over the course of the summer. (It's going to be lots of fun!)

e. **Fledging day minus one (DAY 11)(procedure still to be decided for 95, these instructions from 94)**

On the afternoon of day 11, go to the nest and attempt to count the young. If you are in doubt about the age of the young, please be conservative and go on what you think is day 10. **Do not touch the young**. If it is a nest where you simply cannot see (way back in the roots of a treefall, but sometimes a flashlight helps), then simply note whether the nest is active and state in the nest log that there was no way to be certain of the number of nestlings without risking early fledging.

Assess the situation for catching the young and the adults the next day. Set up a net; if possible, do it in a way that will intercept the adults as they go to the nest to feed the young. Consider the light (i.e., visibility of the net to the parents) in the early morning and make a decision about the best time to return the next day to do the catching. In other words, is this one where you need to get there at dawn if you are to be successful?

f. **Fledging day** (not before DAY 12, later in the year this will be moved up to day 11)

(1) When to go and what to take:

Go in the morning and take the following items:

bird bucket; net, poles, stakes, and hammer (if net is not already set up); fledgling scream tape and tape recorder; and a potter trap;

thermos with ice; stopwatch and bleeding equipment (needles, microhematocrit, clay sealant, critocaps, cotton); optivisor (for mature biologists);

balance, calipers, bands, if necessary, and data sheets.

(2) Begin by catching the adults.

VERY IMPORTANT: When you catch the male and before you do anything else, **CHECK HIS IMPLANT STATUS**!!!! Can you find the implants, how did they appear? Do it **fast**, because the next step is to bleed the birds and we need to do this ASAP.

If at all possible, catch the adults in a way that makes their plasma suitable for hormones, i.e. as they approach or leave the nest with food, rather than after disturbance by you. Bleed them as rapidly as possible and with as little disturbance as possible. Use a stop watch. Keep track of the time of first disturbance, time when they are caught, time when bleeding begins, and time when bleeding is complete. If you catch the birds quickly and can get them bled in 5 minutes, then we can certainly use the blood for hormones. Even two full tubes will allow us to assay for corticosterone, and longer bleeding times are acceptable under certain circumstances. Seal tubes with clay sealant, not with critocaps.

If you are bleeding the bird for CBP (because, e.g., it was quite slow to be caught, so you know it is not useful for hormones), then take your time and get as much blood as possible. Bleeding itself might take 10 minutes, and you might need to prick both wings. Try to get 5 full tubes. Keep notes on the time required for the various steps, even if you are bleeding for CBP. We are interested in both sexes for these assays.

If you simply do not have time to catch the adults this way, they still need to be caught (to be weighed and to check their implant status). To catch them, grab the young (see below) and use them and (perhaps) a tape of fledgling screams to get the adults in a net. This is a less sure-fire method of catching both adults, and the blood may not be good for hormones, unless you catch them immediately, **so this is the less preferred method**.

If the adults have not been bled before, then we must get their blood for DNA as well as hormones or CBP, but ideally this would have been done before fledging day.

Finally, weigh the adults.

(3) Now take the young

THE COUNT OF THE YOUNG IS EXTREMELY IMPORTANT. You also need to weigh them and measure their tarsi. Be extremely alert when you go for the young, or some will slip past you and you will spend lots of time tracking them down. Have a container ready. Approach the nest slowly with your hands in front of you. Extend one or both slowly until you are in a position to strike at the nest the way a snake strikes its prey. When ready, strike with your open hand and cover the nest cavity so that no young can get out. Close your hands over the young and nest. In other words. grab the young and the nest as a unit. Have a back-up person if possible. Put all the young in the same bird bucket (with their parents if you have already caught the parents because parents remain calmer if they are with the young).

After the young are processed, ordinarily you put them back in or near the nest. They won't stay, but the parents will know where to look for them.

If you still need to catch the adults, then put the bucket with young beneath the net. The young will call and the adults should fly into the net immediately. Treat adults as above. If you don't catch them right away, try using the tape. Keep track of time and conditions.

If the adults are too wary to be caught, then process the young. If after having processed them, you still need to capture the adults, you can use the young to catch the adults by putting them in one cell of a potter trap and placing vegetation around it so the only way the parents can get to the young is by entering the trap. Put the trap near the net. Hope.

At some nests we will put the young in open-top containers for a few days and attempt to quantify the feeding behavior of the adults toward their young, according to sex and treatment group. This is a pilot year on this subject, and we don't have the details worked out. Advice is welcome. We have tried paper cartons and hardware cloth. The hardware cloth is not good because the adults try to feed the young through it and the feedings are difficult to count. If we use it we need to put some baffle around the bottom so the parents cannot see the young.

AFTER FLEDGING

still to be decided

1. For the nests where we don't observe adult post-fledging behavior, we need to determine fledgling survival. We always try for survivorship curves (# alive 1 day after fledging, 2 days after fledging, 3 days after fledging, 6 days after fledging, 9 days after fledging, 14 days after fledging). However this is nearly impossible to do. The families move around a lot and you may not find them one day and then find them the next.

2. The day-14 measure is the most important, but you have to visit the territories all along in order to know where the likely places to look are on day 14. So follow Zig's sheets for recording fledgling sightings. Be sure to record what you saw ASAP, so other will not duplicate your efforts.

3. If on day 14 you find all the fledglings that you think are alive, then you can quit looking. If you have not seen one or more of them, keep going to the territories even after day 14, until you are CONVINCED that certain young are not alive. On day 21 you may quit in any case.

Use all your senses. Sound, etc. *The key to success*: Go prepared with information about band colors, so you know what leg and colors to look for. You will get only a few seconds, so preparation will make you much more effective.

4. Return to the lab and record your findings on the fledgling survival sheets. Note where you looked, how much time you spent, how sure you are of your observations, etc.

5. Be on the look out for signs of renesting. Is the female still around the fledglings? Did you see her feed young or just the male? I think that the earliest turn around is a case in which a female laid a second brood egg 8 days after the first brood fledged. But 12 to 15 days is probably more common.

PROCESSING BLOOD AND EMBRYOS

These instructions are minimal because, unlike things that happen in the field, it is easy enough for people to show you what to do. As always, if you are not certain about what to do, ask questions.

(1) DNA

Birds bled for DNA. We need two or three (no more) microhematocrit tubes of blood for each adult and nestling in the study.

Seal tubes with critocaps. Add blood to 0.5 ml of Longmire's solution. USE GLOVES when handling Longmire's.

(2) Hormones and CBP

To process blood for hormones and CBP, the blood should have been sealed with clay sealant. Spin the microhematocrit tubes in the microhematocrit centrifuge for three minutes. **PUT ON THE TOP OR YOU WILL LOSE YOUR SAMPLE**!!!!!!

AND IF YOU ARE DOING MORE THAN ONE SAMPLE BE SURE NOT TO GET THE TUBES MIXED UP. Write down the slot of the centrifuge that each sample goes in.

Take a microhematocrit reading (see below).

Draw off the plasma using a Hamilton syringe that has been rinsed (3 times with distilled water) and dried (bore all the way down, tip placed against a kimwipe, so no chance of dilution). Measure the volume to the microliter with the syringe.

Store the plasma in a well labelled 0.5 ml (i.e., small) eppendorf tube. **Be sure** that the caps are tightly sealed. Freeze the plasma samples. Fill out a bleeding sheet in great detail and indicate on it whether you think the plasma is suitable for hormones or CBP.

If you also need the red blood cells, then after drawing off the plasma, break off the clay seals and blow the red blood cells (not easy) into Longmire's solution using the devise 'designed' for that purpose.

(3) Microhematocrit

For blood collected for hormones or CBP, it is possible to get a reading of the % of blood that is made up of red blood cells. This is known as a microhematocrit reading and is accepted by some as a measure of overall condition. In general (people too), males have higher hematocrit readings than females and castrates have lower readings than intact males.

Blood spun in the microhematocrit centrifuge can be used to take a hematocrit reading.

Preliminary data to date suggest that in late summer, T-males have lower (surprising) readings than controls. We have very few data from earlier in the summer.

To get these readings, line up the swivel reader so that zero is at the top of the clay seal, and 100% is at the top of the column of blood (meniscus at 100%). Then read the % rbc. Don't do this until you are sure of how to do it correctly.

There are data sheets.

(4) Embryos

There is a manila folder and an instruction sheet for how to separate yolk and albumin from embryos in eggs that did not hatch. Tracey (when she comes) and Ellen can give you instruction. It is *critical* that the eggs not break before they are opened and that there be no confusion as to the nest that gave rise to unhatched eggs.

In brief, we need to open the egg over a watch glass and slide the contents away from the shell. We need to add distilled water and/or alcohol to see the embryo. You need to fill out a data sheet and note whether you find an embryo or not. The decision needs to be made at the time the egg is opened. This is not a job that should be postponed because if there is an embryo, it is dead and decaying and we want to preserve it.

If you do find an embryo, please preserve it for DNA. Please keep the upper half, put it in a large eppendorf, label it, and place it down in the -72 freezer. Be sure to wear gloves around that freezer and not to touch any cold surfaces when you are not holding gloves!