# 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).

**MLBS 2003**
**Expanded goals**
**May 6, 2003**

## Core goals

1. Response of T to GnRH challenge in field-caught birds, repeatability and seasonal profile.
2. Stress response and CBG in field.
3. Egg steroids in field.
4. Effect of testosterone implants on CBG, stress series, behavior, and immune function in testosterone-treated and control female juncos in captives.
5. Parental behavior in field and effect of temporary removal of partner of parental behavior of mate as a function of tail white (Joel)

## Only "if possible," more likely for another year

6. Relate behavioral responses of adults to GnRH/testosterone
7. Determine whether fledglings and juveniles respond to GnRH challenge. Find just a few nests off study area (or nests that Joel is through studying) and inject young with GnRH.
8. Hand-rearing juncos to serve as future breeders.

## Overview

- Early Season, bait, then capture juncos with nets and traps, conduct GnRH challenge by bleeding at capture, injecting GnRh, and bleeding again after 30 min.
- In addition, process juncos in normal way (band, sex, age, body size, plumage, fat, condition, etc., see separate instructions) and collect blood for DNA (see separate instructions).
- Finally, Joel will use spec to quantify coloration.
- For birds caught off study area we need a minimum of 30-40 females for Devin (minimum of15 T and 15 C, safer with more).
- Early season, if recapture a bird a second time one week after the first, conduct a second GnRH challenge for comparison with first.
- All season, collect egg 3 or largest for yolk steroids from any nests found with fresh eggs prior to incubation (see separate protocol)
- During nestling phase, when Joel removes females and males to measure parental behavior, challenge removed adults with GnRH.
- At nest-leaving capture male and female for stress series.
- End of season, challenge adults with GnRH.

## 1. GnRH challenge (skeleton, Joe Casto has more detailed protocol)

- Capture birds at nets and traps at any time of day. To determine resting concentration of testosterone in the blood, take a 125ul blood sample immediately after capture by pricking the wing vein (50 ul for T, 150 ul for DNA).

- Inject bird (intramuscular in the pectoral muscle using a 50 ul Hamilton syringe) with 50ul of a phosphate buffered solution (PBS) containing chicken gonadotropin releasing hormone (sigma chemical, c-GnRH-I, concentration, 1.25ug/50ul PBS).
- Place bird in a paper bag.
- After 30 min, take a second 100ul blood sample (for T and for cort).
- Release the bird.
- Aim is to challenge 200 adult juncos, 100 males and 100 females [More likely we will achieve 100 adults, 50 of each sex].
- Later in season, may elect not to return bird to lab, conduct GnRH challenge in the field.
- Materials needed, GnRH solution kept cold, 26 gauge needle to collect blood sample, 50 ul Hamilton syringe for injection, stop watch for precise timing, freezer to keep GnRH frozen until use, ice to keep blood samples cold until they can be spun and plasma collected. For second challenge will collect 50ul for first sample, 100ul for second sample.
- Where possible adult birds will be sampled a second time soon after the first in order to determine repeatability of response within a stage of reproduction and a second time at a later stage of reproduction (incubation/nestlings)(minimum of 48 hrs between challenges, collect only one tube for basal and one tube for 30min sample0;
- Nestlings will be challenged, if at all, only once at 11 days of age (is it reasonable to expect a response from a nestling?)

**2. Stress series (more detail in separate protocol)**

- Using similar methods to the GnRH challenge we will measure the rise in plasma corticosterone in response to a mild stressor, which is capture and handling, bleeding at time 0, 15 min, and 30 min.
- We will later determine whether response to a stressor co-varies with the response to the GnRH challenge, i.e., do individuals that respond to GnRH with large increase in testosterone respond to handling with a large or small increase in corticosterone?
- Birds will be captured with traps and nets and bled (50 ul blood) immediately upon capture (within 1 minute).
- They will be placed in cloth bags and bled again after 15 min and 30 min (50 ul blood each time).
- Birds will then be released.

**3. Egg steroids (more detail in separate protocol)**

- To determine whether the testosterone content of an individual's eggs co-varies with the response to a GnRH challenge, we will collect 1 egg from the clutch of each female and determine yolk steroid concentrations. Typically we will collect the third egg laid from 3 or 4-egg clutches. Steroid content at laying can only be determined from fresh eggs prior to the onset of development.

**4. Effect of testosterone implants on female behavior and physiology**

- As part of our ongoing studies to compare the effect of testosterone on males and females, we will compare captive, testosterone-treated and control females (T- and C-females) for their rise in corticosterone in response to handling ("stress series"), their levels of corticosteroid binding globulin (CBG), and their response to challenges to the immune system. These studies are part of larger experiment to compare males and females by asking whether attributes already known to be testosterone-sensitive in males (where T-sensitive means that an attribute of behavior or physiology is affected by treatment with testosterone) are also T-sensitive in females.

- The experiment will compare 15-20 T-females to 15-20 C-females and will be carried out at MLBS.

- The females in the experiment will be captured in early spring in Virginia before they have begun to reproduce on their own.

- Will compare captive T- and C-females for stress series, CBG, and immune response as part of larger experiment to determine which attributes are T-sensitive in both males and females.

- Might also use GnRH challenge as a way of determining effect of T on female behavior - compare behavior of female pre- and post-challenge with GnRH (a la Creagh and mealworms, each bird as it's own control), e.g., activity levels, response to intruder. This is a good sub-project for Devin's study of captives because birds are there and results are visible without waiting for the assays, but the experiment is already busy.

**5. Relationship between a male plumage character and parental behavior**

- The question is whether plumage traits in juncos can be used by other juncos to predict the parental ability of both males and females.

- Males and females will be captured, banded, and measured. We will quantify a plumage trait known as tail white by eye and also by photographing the bird's tail.

- We will follow the subsequent reproductive success of the pairs that form.

- A t the time that the female is incubating we will place a temperature sensor in her nest and film her behavior in order to quantify when she begins to incubate and how steady her incubation behavior is. We predict that females with brighter plumage will be better incubators (start sooner, spend less time off the nest, hatch their young earlier).

- At the time that a pair has a nest with young that are between 3 and 7 days of age, we will capture the male or the females at the nest and hold him or her for one half day.

- We will observe behavior at the nest of the pair member that remains in the field while we are holding the mate in captivity. We will quantify behavior in response to the pair member's absence (number of feedings, song rate).

- The following day we will capture the other pair member and hold it for one-half day and observe the behavior of the other pair member in the field. Temporary removal of one parent is necessary to simplify the assessment of the remaining parent's response to the offspring.

- We predict that individuals with better-developed plumage characters will compensate fully for the partner's absence while individuals with less developed characters will only partially compensate.

- The adults and offspring will not be harmed because the period of detention is brief and the other parent is expected to compensate.

**6. Does GnRH/testosterone relate to (alter) behavior of adults? (postpone for another year?).**

- Two related questions are (1) does the increase in testosterone stimulated by GnRH injections alter behavior during the time testosterone is elevated, or (2) does a bird's response to a GnRH challenge predict undisturbed behavior measured at another time? To answer the first question we would measure behavior, inject with GnRH, and measure behavior again. In the second case, we would inject with GnRH at one time and measure behavior under controlled conditions at another time.

- One way to ask whether testosterone affect adult behavior is to measure the effect of a GnRH (and its known effect on testosterone) on levels of activity in captive adults. We might, for example, observe behavior in caged birds for ~20 minutes (time to be adjusted depending on preliminary trials) and assess activity as the number of perch changes. We might then inject the bird with the same dose of GnRH as used in the challenge (see above), wait 45 min and measure activity again for another 20 minutes. Controls would be given an injection of vehicle (phosphate buffered saline, PBS) and otherwise observed in the same way. Study could involve 20 males and a paired design in which we measure response to the control injection followed by response to the GnRH injection.

- To make the measure more ecologically relevant, we might measure level of perch hopping in response to playback of nestling begging calls. Experimentals would receive a GnRH injection; controls would receive a PBS injection. As before, birds would be housed in cages, but the experiment could be conducted outdoors near active nests. We anticipate that birds with nestlings will become hyperactive when played nestling vocalizations. If testosterone interferes with parental behavior in females as it does in males, we anticipate that females given a GnRH injection will be less affected by the playback (hop less frequently). In sum, the procedure would require that we find an active nest, capture the junco as it comes to feed the young, inject it with GnRH or PBS and observe behavior near the nest. We will then release the bird. This experiment is probably not compatible with Joel's work and would need to be conduced off the study site.

- Response to Simulated Territorial Intrusion (STI)(same rationale, different stimulus).

**7. Does GnRH/testosterone relate to (alter) behavior of nestlings? (postpone for another year?).**

- Many studies have shown variation among bird eggs in the concentration of testosterone in the egg yolk. T varies among eggs within a clutch and among females regardless of the order in which an egg is laid. Several investigators have speculated that yolk testosterone makes the offspring better competitors for food. Very few studies have asked whether nestlings are able to produce testosterone on their own and none to our knowledge have tested whether testosterone affects nestling competitive behavior, in particular frequency or intensity of nestling begging calls.

- We will measure the effect of testosterone on nestling begging behavior by injecting with GnRH (or testosterone) or vehicle (PBS, oil) and returning the nestlings to the nest.

- When young are 6-8 days old, we will remove two randomly chosen nestlings from the nest and introduce them into comfortable false nests. Broods consist of 3-4 young, so the parents will continue to feed the young that are left behind.

- The dose administered to nestlings will be half that given to adults (1.25 ug/50ul of phosphate buffered saline). One nestling will be randomly selected (coin flip) as a control, the other as the experimental.

- Every 15 min for one hour and 15 min, we will stimulate the young in the way parents would if they were bringing food (tap the side of the nest bowl and gently tap the nestling's bill. We will measure the extent of the nestling's reach and record its vocalizations.

- In a paired set of presentations (different nestlings different nests or same nestlings on the following day), we will offer the nestlings food at the same intervals and measure the amount of food they consume.

- After the observations we will return the nestling to its nest. Prior experience with this method on this population has shown that no harm is done to the nestlings.

**8. Breeding juncos in captivity/measuring response to GnRH challenge in siblings**

- Some studies are better performed on captives and it has been a long-term goal to breed juncos in captivity. Each year we come a little closer to the goal, and we will continue with our efforts to breed juncos in captivity at KFBO this summer.

- Having consulted with experts in Europe, the single best predictor of whether songbirds will breed as captives is whether they have been reared in captivity. It seems to be an

issue of fear – hand-reared birds are less afraid of caretakers, loud noises, etc. and this seems to enhance their performance.

- We too have observed that the few juncos we have reared in Bloomington are more ready to breed than birds captured in the field.

- We request permission to capture and transport to Bloomington 60 sibling juncos, 30 males, 30 females, taken from the nest at 8-10 days of age. We have done this before and know what is required. Young need feedings several times an hour for a period of 2 weeks. Food will consist of canned cat food, live moths, powdered calcium, boiled eggs, ground carrots, powdered bird food, and vitamins. We will have a team of 7 people to share the work and know from experience that this can be done successfully.

- Once returned to Bloomington, we will bring the young to adulthood, measure response to GnRH challenge to see whether relatedness is a key variable.

- They can then serve as parents for the next generation of juncos in Bloomington.

**9. GnRH responses in other species (another year)**

- Bird species differ in the degree of difference between males and females. They also differ in the levels of testosterone found in females. One hypothesis predicts that testosterone is similar in species in which the sexes look alike and dissimilar in species in which they differ.

- We will apply the method of the GnRH challenge to males and females of a number of species commonly nesting at MLBS and at KFBO in order to see whether the sexes are similar or dissimilar in the way in which they elevate testosterone in response to GnRH. We have selected species pairs, one from Virginia and one from Indiana, that are dimorphic: northern cardinals IN), rose-breasted grosbeaks (VA), and robins IN and VA) vs. a species pair in which males and females look alike, chipping sparrows (VA) and field sparrows (IN).

- Other that the difference in species, other methods will be the same as those already described in terms of methods of capture and dose.

**Other projects under consideration for other years**

1. Consider question of disadvantages of T to females and determine whether T-females are more or less attractive than C-females to males and whether they are dominant over C-females, and especially whether status is inversely proportional to attractiveness as preliminary data suggest. This would be an extension of Ian's work and is something we promised to do for the grant.

2. Tail white experiments re development of tail white in nest, relationship between tail white and parental behavior in adults and in young (Joel has ideas here)

3. GnRH challenge and nestling begging intensity, does gonad respond? That is, does induction of T in nestlings increase growth or begging or both? Fits in nicely with all the maternal effects work being done on nestlings? Is nestling behavior affected? (REU project? Doable in conjunction with Joel?)