

## 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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## **GnRH Challenge Protocol**

**Written by Joe Casto, 2003, modified slightly by JWM and EK, 2004)**

1. Thaw a vial of GnRH. Each vial contains 500  $\mu\text{l}$  of GnRH solution (500 ng/20  $\mu\text{l}$ ). The first vial will be enough for 7-8 birds (due to priming the syringe), each subsequent vial will do approximately 9 birds.
2. Prepare a syringe as follows:
  - Using a disposable 1 cc syringe with needle attached draw up 0.2 cc of solution from the vial.
  - Hold the disposable syringe with the needle pointing upward and draw in 0.2 cc of air.
  - Flick the syringe with your finger to get the air bubbles to the top. [note: helps to flick hard below where the liquid is.]
  - With the needle still pointing upward, eject the air until a drop of liquid appears at the tip of the needle.
  - Take the plunger out of the 50  $\mu\text{l}$  Hamilton syringe (glass-tipped, the kind to which you add a needle).
  - Make sure there is no liquid in the barrel of the Hamilton syringe. Use the disposable syringe to fill 1/3 of the Hamilton syringe from the back end. (make sure there are No air bubbles). [It helps to hold the Hamilton in your left hand with a slight upward tilt and use your right hand to place the tip of the needle of the disposable syringe into the barrel of the Hamilton bevel side up.]
  - Use the plunger of the Hamilton syringe to push the solution through the Hamilton syringe and eject the GnRH solution back into the vial. Now there should no longer be an air space at the front end of the syringe.
  - Eject the rest of the GnRH solution out of the disposable syringe back into the vial.
  - Now put the tip of the Hamilton syringe into the vial and fill the syringe with fluid (there should be no air bubbles).
  - Put a clean 26 g 1/2 inch needle on the Hamilton syringe and eject its contents back into the vial.
  - You now have a Hamilton syringe that is ready to load for GnRH injections (no air bubbles).
3. Fill the syringe with 50  $\mu\text{l}$  of GnRH solution.
4. Collect a pre-challenge blood sample (125  $\mu\text{l}$ ) from the alar wing vein. *[note change from last year when we were collecting 50 ul. This could matter if it takes longer to collect 125 ul than 50 ul.]*
5. Using an alcohol swab, wipe the pectoral muscle of the bird, this will make the feathers easier to move out of the way.
6. Locate the pectoral muscle, using your thumb pull the skin to one side, insert 1/3 - 1/2 of the needle into the pectoral muscle approximately 2-3 mm lateral to the keel of the sternum, inject the GnRH, remove the needle, and let the skin slide back in place. **Record the time of injection.** *[If the GnRH solution leaks back or the bird bleeds, make a note.]*
7. 30 min following the challenge injection, collect a post-challenge blood sample (100  $\mu\text{l}$ ).  
**Record the time the blood sample was collected.** *[note change from last year when we were*

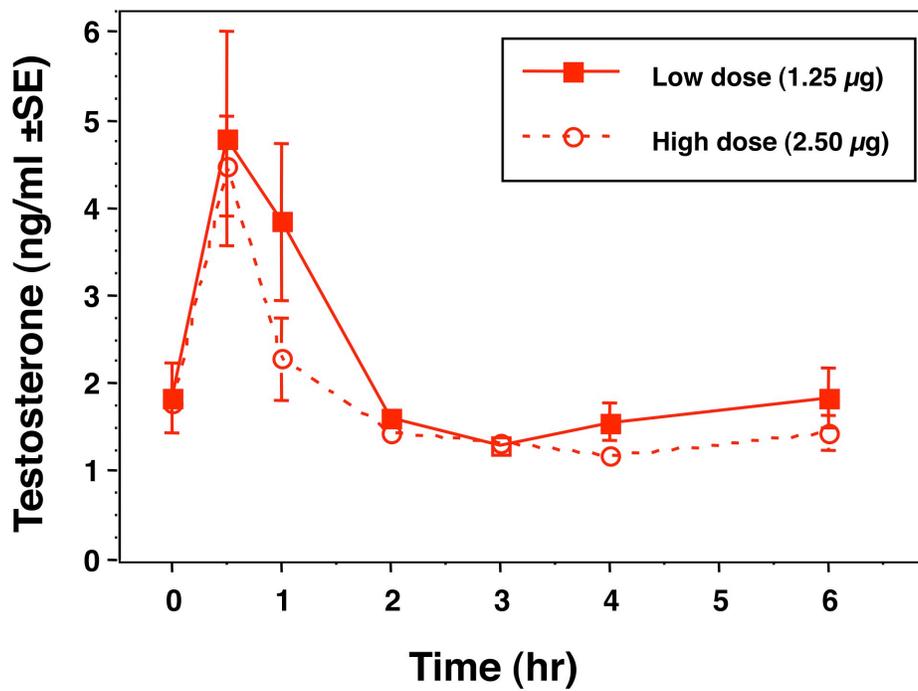
*collecting 50 ul. This could matter if it takes longer to collect 100 ul than 50 ul.]*

8. If you are injecting many birds, wipe the needle with an alcohol swab between injections, but use the same needle to avoid having to evacuate air bubbles from new needles before every injection.

9. Once finished, discard the needle and rinse the Hamilton syringe several times with distilled water.

10. [New note for 2004: After spinning blood (5 min in centrifuge), draw off and save plasma. If DNA sample is needed, break off the ends of the hematocrit tubes that contain only the cellular fraction. Immediately eject the cells into Longmire's solution using a plastic syringe. [in 2004 blood was refrigerated in microhematocrit tubes until after all the birds had been challenged and bled in the AM or the PM, but then spun immediately. No worms formed, samples look good, but important Not to let samples sit for long before spinning. Plasma for IgG (Tim) and testosterone was stored separately.]

## RESPONSE TO INTRAMUSCULAR c-GnRH-I CHALLENGE, Males



Sample sizes: 0 hr = 5 each; 0.5 - 6 hr = 3 each