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).

Revised April 22, 1997

PROCESSING BLOOD

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 only, 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. (See detailed instructions posted in lab).

(2) Hormones and CBG

To process blood for hormones and CBG, the blood should have been sealed with clay sealant. Spin the microhematocrit tubes in the microhematocrit centrifuge for three (check this against other instructions, may be 5) 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.