Indiana University Bloomington IUScholarWorks

## **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 (<u>https://scholarworks.iu.edu/dspace/handle/2022/7911</u>).

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For additional information, visit the Ketterson/Nolan Lab community on the <u>IUScholarWorks repository</u>

## **Spectrometer Protocol**

- 1) Turn on deuterium-tungsten lamp light source to let the light source warm up for at least 30min prior to samples.
  - \*Xenon pulse lamp does not need to be warmed up.
- 2) Open Ocean Optics Program (OOI base 32) (<u>www.oceanoptics.com</u>)
- 3) Set the average and boxcar (typical #s are average = 20, boxcar = 10) but for
  \*Xenon pulse lamp you should probably use an average = 50, boxcar =10)
- 4) Set distance of probe to sample (typical distance = 1 to 5mm)
- 5) Place the probe on the white standard and then set the 'integration time' so that there is a peak scope score of 3500-3800. \*The Xenon pulse lamp: the integration time determines the speed of flash, if it flashes too fast it will heat up and the bulb will not last. Set integration time and distance to probe such that the pulse it no faster that each 0.5sec
- 6) Set your black standard by 1) turning off the light or 2) putting probe in 'black box'. Clicking 'Black light bulb' button.
- 7) Click the 'negative black light bulb' to subtract all light
- 8) Set your white standard by placing the probe on white standard, turn 'light on' and click the 'yellow light bulb'.
- 9) Change the Y axis to transmission mode (reflectance mode) by clicking on the 'T' button.
- 10) Change the axes by going to 'View', then 'Spectrum scale', then 'Set scale' to see 300 to 700nm and 0 to 110% light.
- 11) Choose the folder that you want to put the data in (make folders in 'explore')
- 12) Go to 'file', 'save', 'processed' file. Name first one 'practice' and save as a 'processed' file to set the default saving mode to processed.
- 13) Use automated save by going to 'file', 'automated save', 'base name' --Name it by the drive, and folder that you want the data in (ex C:\flowers\2006data).
- 14) Set the extension number going to 'file', 'automated save', number' starting with 0001.
- 15) Click 'enable' to make it work and click 'show name' to see the next name.
- 16) Between samples, place the probe on the white standard and click 'yellow light bulb' *And* rename the 'base name' and the 'starting number'.
- 17) Be careful with white standard- (if it gets dirty there is a protocol to clean it with sandpaper and nitrogen gas, see Ocean Optics)

### Trouble shooting

Setting up spectrometer with PC for the first time:

1. Always install OOI before connecting Spectrometer to PC.

2) When using the S2000 + AD converter for the first time with a new PC, you must configure the software for the spectrometer. Go to 'Spectrometer', 'Configure' input data for serial number and the gratings for the spectrometer. This information is available from Ocean Optics (727)-733-2447.

3) When using the USB2000 for the first time with a new PC, you might need to configure the software for the spectrometer. Go to 'Spectrometer', 'Configure' input data for serial number, go to A/D interface and choose S2000/PC2000/USB2000/HR2000.

Light in the Counts Mode is abnormally weak

- 1) fibre optic may be connected backward (attach end with 5 fibers to the light source and end with 1 fiber to spectrometer.
- 2) Make sure fiber optic cord has not been bent, fibers broken.
- 3) Make sure white standard is clean.

Inconsistent readings

- Fiber optic cord may be bent, fibers broken.
- White standard may not be clean
- DTA Light source may not have warmed up enough (light intensity increases with heat). Re- save the white reference and redo measurements
- Xenon pulse lamp may be overheating. Let it cool and start again. Make sure pulse rate is not very fast.

Spectral data is very noisy

Boxcar may be set too low (use 10) or use Montgomerie's program 'SmoothR1.7'

All spectral curves have a small abnormal 'peak'

This is a saturation point and is normal. You can smooth over this peak by using Montgomerie's program 'SmoothR1.7'

Data does not result in 2 columns, one for wavelength and one for reflectance You have saved data in the wrong mode. Change to 'processed', save a practice and then change to Autoincrement file name.

Xenon pulse lamp will not turn on:

Make sure that the Strobe/lamp enable window is clicked 'on'. Make sure the spectrometer, light source, and computer are properly connected (see PX2 operating manual (<u>www.oceanoptics.com</u>).

Make sure the 'on' switch and single vs multiple switch is correct. May need a replacement light bulb – order from OO.

# **Color Analysis**

http://biology.queensu.ca/~color/ This website has links to:

Bob Montgomerie's spectral processing program ColoRv1.7.

Koederitz's spectral processing program Spectre

Hadfield's program **SPEC** that calculated photo catches based on cone sensitivity data, three different irradiance specta (daylight blue sky, and forest shade), and the transmission spectra of some ocular media (blue tit, chicken, and human). Also calculates the discriminablity of two stimuli based on the receptornoise-limited color opponent model of Vorobyev et al. 1998.