Date: 12-15-2020

IRB #: FY19-20-238
Title: Comparing fecal sac dissection with DNA barcoding in determining nestlings' diet
Creation Date: 12-19-2019
End Date: 1-19-2023
Status: Approved
Principal Investigator: Virginie Rolland
Review Board: IACUC (Institutional Animal Care and Use Committee)
Sponsor:

Study History

Submission Type Initial	Review Type Full	Decision Approved

Key Study Contacts

Member Virginie Rolland	Role Principal Investigator	Contact vrolland@astate.edu
Member Virginie Rolland	Role Primary Contact	Contact vrolland@astate.edu

Initial Submission

A. PI Contact Information

Primary Contact

A.1. Browse to select the primary contact for this study.
Typically, this is the PI. *This field is required by the CayuseIRB software.*

Name: Virginie Rolland
Organization: Biological Sciences
Address: P.O. Box 599 , State University, AR 72467-0599
Phone: 870-972-3194
Email: vrolland@astate.edu

If you cannot find the Primary Contact using the "Find People" button above, click here for instructions on requesting Cayuse credentials.

Principal Investigator (PI)

A.2. Browse to select the PI for this study.
This field is required by the CayuseIRB software.

Name: Virginie Rolland
Organization: Biological Sciences
Address: P.O. Box 599 , State University, AR 72467-0599
Phone: 870-972-3194
Email: vrolland@astate.edu

If you cannot find the Principal Investigator using the "Find People" button above, click here for instructions on requesting Cayuse credentials.

Attach PI's CITI Certificates:

- At a minimum, these parties must complete the most relevant Responsible Conduct of Research course. Other courses may apply, e.g., Human Subjects Research course for IRB protocols, Working With the IACUC course for IACUC protocols, or Biosafety/Biosecurity course for IBC protocols.
- To complete these courses, please create an account at www.CitiProgram.org. You must affiliate with Arkansas State University when you register.

A.3.

- To obtain a PDF of the completion report, log in to CITI, select Arkansas State University Courses, select View Previously Completed Coursework, select View under the Completion Report column for the course, right-click on the report, and save as a PDF file.

IACUC certificate expires 7.19.21.pdf

Rolland IACUC_citiCompletionReport_6.30.2018.pdf

A.4. **PI's relationship to Arkansas State University**

✓ Arkansas State University - Jonesboro

✓ Faculty

Staff -- If you are conducting this research as a student, please select your student status.

Post Doc

Graduate Student

Undergraduate Student

Other (e.g., ASU-MH, NYIT, or Catalyst entities)

A.5. **Co-Principal Investigator(s)**

Does this project involve Co-PI(s)?

Yes

✓ No

A.6. SPONSOR

Is this project funded by a grant, internal or external?

Yes

✓ No

A.7. COMPLIANCE COMMITTEE SELECTION

Select the appropriate committee for this protocol.

Institutional Review Board (IRB) -- for research involving human subjects

✓ Institutional Animal Care and Use Committee (IACUC) -- for research involving animals

Institutional Biosafety Committee (IBC) -- for research involving recombinant DNA or other potentially hazardous biological materials

B. IACUC Protocol

PLEASE NOTE: THE FIRST USE OF EACH ABBREVIATION OR ACRONYM MUST INCLUDE THE DEFINITION.
Example: APHIS (Animal and Plant Health Inspection Service)

RESEARCH OR SOP

Are you submitting a research protocol or a standard operating procedure (SOP)?

✓ Research Protocol

Standard Operating Procedure (SOP)
-- Typically submitted by the Animal Facility Manager

B.1. EFFECTIVE DATES

Expected Start Date
04/01/2019

Expected End Date
03/31/2022

SPECIES

B.2. Eastern Bluebird (Sialia sialis)
Carolina Chickadee (Poecile carolinensis)
Tufted Titmouse (baeolophus bicolor)
Carolina Wren (Thryothorus ludovicianus)

B.3. STRAIN/STOCK

N/A

B.4. SOURCE

From the wild

B.4.a. In-house breeding colony?

Yes

✓ No

B.5. SEX

✓ Male

✓ Female

B.6. TOTAL NUMBER OF ANIMALS TO BE USED

155

B.7. MAXIMUM NUMBER OF ANIMALS TO BE HOUSED AT ONE TIME

0

RATIONALE FOR RESEARCH USING ANIMALS

B.8. Please justify this research, briefly explaining whether it is new research, why the research is important, and why it is necessary to use animals.

Only a brief description is necessary. Do not cut and paste your grant proposal here.

The objective is to determine prey fed to nestlings from fecal sacs. Many techniques have been used to determine prey, including fecal sac dissection (Van Horne and Bader 1990, Kleintjes and Dahlsten 1992, Moreby and Stoate 2000), ring collar (Orians and Horn 1969, Pinkowsky 1978, Bay and McGaha 2000, Moreby and Stoate 2000), photography/video and observations (Pinkowski 1978, Kleintjes and Dahlsten 1994, Bay and McGaha 2000), regurgitation (Carlisle and Holberton 2006), and gut content analysis from euthanized animals or individuals found dead (Kleintjes and Dahlsten 1992). Regurgitation, gut content analysis, and ring collar are invasive techniques and nowadays mostly abandoned for less invasive and more humane techniques. Photography/video requires expensive equipment and, even with high resolution, this technique does not always work depending on the angle of the camera relative to the parent feeding the nestlings. Fecal sac dissection is another non-invasive technique that has proved successful at least for some species (Van Horne and Bader 1990, Kleintjes and Dahlsten 1992). However, some prey are more easily digested than others. For example, soft-bodied caterpillars are more easily digested than beetles; whereas beetles leave body parts undigested, nothing remains of the caterpillars. This differential digestion leads to biased results. Additionally, Dr, McKay (entomologist) and I previously conducted a prey-sampling study using fecal sacs on bluebirds, and fecal sacs had revealed nothing, as though bluebird nestlings were completely digesting their prey. However, we were only collecting fecal sacs from nestlings that were 14 days old and it is possible that at this advanced nesting age, their digestive system is more effective at digesting. In addition, studies published on fecal sac dissection had been conducted on other species than bluebirds. Therefore, this time, I intend to sample nestlings of not only bluebirds but also tufted titmice, Carolina chickadees, and Carolina wrens at various ages. Finally, a more recent technique has emerged: DNA metabarcoding (Jedlicka et al. 2016, Trevelline et al. 2018). Although DNA barcoding cannot be used to quantify the number of individual prey, this technique allows one to identify prey in a "soup" of various species to the species level. My project will compare fecal sac dissection with DNA barcoding in identifying prey of nestlings. Speaking with Dr. Trevelline (expert in DNA barcoding of prey in bird and rodent feces), this study has never been done. As the climate changes, insect communities are expected to shift, which would influence the

diet of their consumers, such as birds. To monitor these changes and how birds may be impacted, it is important to accurately determine their diet.

RATIONALE FOR USING SELECTED SPECIES

B.9. All selected species are insectivorous songbirds that readily use manmade cavities, such as nest boxes, to build their nest. I already monitor a trail of nest boxes for the study of bluebird population dynamics, so the added disturbance for fecal sac sampling would be minimal at those nest boxes compared to sampling fecal sacs from nestlings outside this nest box trail. Finally, primers for similar prey already exist (Jedlicka et al. 2016).

EXPERIMENTAL/INSTRUCTIONAL PROCEDURES

Please describe the experimental/instructional procedures. In cases where offspring are used in experiments, what are the plans for the mothers within the study?
Please provide a general description of the experiments and the experimental design. Do not cut and paste your grant proposal here.

B.10. Nest boxes are checked every 1-6 days for nesting activity. I record the nest stage (e.g., nest building, egg laying, incubation, number of chicks) at every visit, regardless of the species occupying the nest box. In addition to the monitoring procedure described in protocol #FY17-18-484, I will collect fecal sacs from bluebird, tufted titmouse, Carolina chickadee, and Carolina wren nestlings at 4, 8, 12, and 16 days old. Although nestlings frequently defecate when handled, this does not always happen. If a nestling does not release a fecal sac naturally, I will gently pull on its cloaca to facilitate the release of its fecal sac (Trevelline et al. 2018). The collected fecal sacs will be placed in 20-mL vial filled with 15 mL of 95% ethanol and the nestlings will be placed back in their nest. Samples will be stored in -80°C freezer until dissection and between dissection and DNA extraction. Therefore, time at any given nest box should not exceed 10 min. The fecal sacs will be dissected in the lab under a dissecting microscope and any body part will be identified to the lowest taxonomic level possible, using the key by Triplehorn and Johnson (2015). Dissecting instruments will be placed in 10% bleach for 30 seconds between batches of samples coming from siblings of the same nest age to avoid cross-contamination. Dissected fecal sacs will be placed back in a tube with ethanol until extraction. For extraction of the prey DNA, we will use an extraction kit, such as Xpedition Soil/Fecal DNA MiniPrep Kit D6202 (Zymo Research) or QIAmp DNA Stool Mini Kit (Qiagen) following protocols by Jedlicka et al. (2016) or Trevelline et al. (2016, 2018) before shipping to the University of Illinois - Chicago for prey DNA sequencing.

Click below to add attachments, such as PDF files containing diagrams, etc.
Please identify each attachment as it relates to the description above, e.g., "Figures 1 - 4."

ANIMAL PROCEDURES

Please provide a description of all animal procedures, surgical and non-surgical.

B.11.

- To justify the number of animals that will be used, provide a statistical analysis, such as power analysis, and include the result of that analysis in the description.
- With the description, please include a table that includes group, treatment, number of animals, pain category, and whether the animal will be euthanized at the end of the experiment. (A sample is attached below.)

IACUC 2019 protocol_Section B11.docxSample documents: Sample Animal Procedures.xlsx

B.12. **USDA PAIN AND DISTRESS CATEGORIES AND CONSIDERATION OF ALTERNATIVES**

B.12.a. USDA CATEGORIES FOR PAIN AND DISTRESS

Check all that apply

Category B: No pain or distress

Animals being bred, conditioned, or held for use in teaching, testing, experiments, research, or surgery, but not yet used for such purposes.

Examples include:

- breeding colonies
- wild animals held for observation

Category C: Slight or momentary pain or distress or no pain or distress

Animals upon which teaching, research, experiments, or tests will be conducted involving no pain, distress, or use of pain-relieving drugs.

Examples include procedures performed by correctly trained personnel, such as:

✓

- blood collection from a common peripheral vein per standard veterinary practice
- parenteral injections of non-irritating substances
- euthanasia by IACUC approved methods
- short term manual restraint

Number of animals subject to Category C

155

Category D: Pain or distress appropriately relieved by analgesia, tranquilization, or anesthesia.

Animals upon which experiments, teaching, research, surgery, or tests will be conducted involving accompanying pain or distress to the animals AND for which appropriate anesthetic, analgesic, or tranquilizing drugs will be used.

Examples include:

- surgical procedures
- blood collection by more invasive routes
- administration of drugs, chemicals, or toxins that would be expected to produce pain unless alleviated by analgesics

Category E: Unrelieved pain or distress

Animals upon which teaching, experiments, research, surgery, or tests involving accompanying pain or distress to the animals will be conducted AND for which the use of appropriate anesthetic, analgesic, or tranquilizing drugs will adversely affect the procedures, results, or interpretation of the teaching, research, experiments, surgery, or tests.

Examples include:

- toxicity studies
- microbial virulence testing
- research on stress, shock, or pain
- negative conditioning via electric shocks that would cause pain in humans

EUTHANISIA

B.13.

Are animals to be euthanized at the end of the investigation?

Yes

✓ No

B.14. **Which approved animal facility euthanasia protocol(s) will be used?**
Include the protocol number and title, if possible.
If there is only one approved animal facility protocol available, you are expected to use that.
(Please check with the animal facility manager at (870) 680-4855.)

N/A

B.15. **HAZARDOUS AGENTS**

Will animals be exposed to hazardous agents?

Yes

✓ No

B.16. **ANIMAL DISPOSAL**

Recommended disposal route

Incineration

Other

Please attach any other pertinent documents here.

Rolland_Federal Banding Permit - expires 9.20.2021.pdf

Rolland2020.pdf