

Revised

NOV 29 2018

College of Natural Resources & Sciences
Humboldt State University

HUMBOLDT STATE UNIVERSITY

INSTITUTIONAL ANIMAL CARE AND USE PROTOCOL FOR THE HUMANE CARE AND USE OF LIVE VERTEBRATE ANIMALS

**This box is for the review of the use by the Institutional Animal Care and Use Committee.
Authors should not write or type inside the borders of the box.**

Date 1st Received: 11/13/18 Revision 1 Date: 11/29/18 Revision 2 Date: _____ No. 18, 19, B, 40 - A

- ☐ E- Procedures are exempt from full IACUC review because they are purely observational, non-invasive, and produce no perceptible discomfort or they concern only the use of tissues from dead animals. To be considered exempt, tissues from dead animals must be obtained from animals euthanized or otherwise killed by means, and for purposes, unrelated to the proposed project. The procedure may be approved by the Chair one additional member of the IACUC.
- ☒ A- Procedures will be minimally invasive or produce relatively little discomfort. Protocols may involve, bleeding, injections, minimal sampling, anesthesia or humane euthanasia without prior invasive manipulation. The procedure may be approved by the Chair and two additional members of the IACUC. Project topics will be reviewed by the IACUC at the next scheduled meeting.
- ☐ B- Procedures will involve prolonged manipulation or be invasive. Protocols may involve surgical or other stimuli inducing pain or distress, but all pain or distress will be mitigated with appropriate anesthetics or analgesics. The procedure may be initially approved by the Chair, the Campus Veterinarian and one additional member of the IACUC. Protocols will be reviewed by the IACUC at the next scheduled meeting.
- ☐ C- Procedures will be invasive and may cause prolonged physiological or psychological stress. Pain, considerable distress, or discomfort may be induced and not mitigated by anesthesia or adequate analgesia (e.g. LD50 experiments, long-term food or water deprivation, etc.). These protocols will be reviewed thoroughly by the IACUC prior to commencement of the project.

Requires Health Assurance ☐ Yes ☐ No

see attached email 11/30/18
Signature, IACUC Member Date

☒ Approved ☐ Denied

[Signature] 11/30/2018
Signature, IACUC Member Date

☐ Approved ☐ Denied

Signature, Campus Veterinarian (if necessary) Date

☐ Approved ☐ Denied

[Signature] 12-6-18
Signature, IACUC Chair Date

☐ Approved ☐ Denied

Final Committee Decision. All protocols must be approved prior to the start of research.

Protocol Edition 2/15/2017

1. **Faculty Project Leader:** Melissa Hawkins

Department Affiliation: Biological Sciences Department

2. **Project Title:** Elucidating the relationship between the Humboldt and San Bernardino flying squirrels using conservation genomics

3. **Email address(es) of the Faculty Project Leader and other corresponding applicants:**

Melissa Hawkins: mtr57@humboldt.edu
Stella Yuan: scy8@humboldt.edu

4. **Names of others handling live animals in the absence of, or not directly supervised by, the faculty project leader, and their qualifications to perform the procedures indicated. (Do not include class rosters here - see 8 below):**

Direct handling of live animals will not be necessary for this project.

5. **Will the described project be funded?** ☒ Yes ☐ No

If funded, will the funds be administered by the HSU Sponsored Programs Foundation (SPF)?

☒ Yes ☐ No

If funded, but not administered by the HSU SPF, then list the unit that will administer the funds:

Click or tap here to enter text.

6. **Proposed starting date (the starting date cannot precede date of approval, and all protocols must be renewed or extended annually).** The Annual Protocol Review Form must be approved on or before the anniversary of the approval date to indicate termination of the project or to request extension of the dates of approval.

Jan. 22, 2019

7. **Provide a brief, non-technical, description of the project. Your response should include the proposed goals, general methods, and educational or scientific objectives that the proposed use is designed to meet.**

This project is a part of Stella Yuan's master's thesis, and the goal is to compare the genetic diversity between the Humboldt flying squirrel and its subspecies the San Bernardino flying squirrel. Because the San Bernardino flying squirrel population is isolated and cut off from other flying squirrel populations, it is hypothesized that they will have low genetic diversity compared to the Humboldt flying squirrel, which may increase the risk of extinction. The San Bernardino flying squirrel was under review to be listed as an endangered subspecies by the U.S. Fish and Wildlife Service, but ultimately rejected. This decision, however, was made prior to any genetic evaluation of this subspecies, so the results of this study could

help inform future conservation decisions. In order to compare genetic diversity, hair samples will first be obtained from current flying squirrel populations by setting hair snare traps in their habitats. Then, we will extract DNA from the hair samples, sequence it, and compare their sequences. Samples from museum specimens will also be taken and sequenced to increase sample size.

8. Is the primary purpose of the project for ☐ instruction, ☒ research, or ☐ both?

Based on your answer, please address the relevant questions below.

If the primary purpose is for instruction, list the course number and write the CRN for this semester (note that this CRN will need to be updated with any future offering of the course covered by this protocol).

Course # (e.g. ZOOL 356): Click or tap here to enter text.

CRN: Click or tap here to enter text.

Will all of the enrolled students in the course denoted by the CRN above participate in the use of animals covered by this protocol? ☐ Yes ☐ No

If no, then provide a list of the students exposed to, or otherwise using, live vertebrate animals.

Click or tap here to enter text.

Describe the learning objectives that justify 1) the use of, and 2) duplication of procedures involving, live animals for instruction.

Click or tap here to enter text.

If the primary purpose is for research, explain how you determined that this protocol does not unnecessarily duplicate previously published observations or experiments; please include:

1. **the type of literature searches conducted:**

PubMed and Google Scholar

2. **keywords used:**

Glaucomys sabrinus californicus genetics

Glaucomys oregonensis californicus genetics

San Bernardino flying squirrel genetics

San Bernardino flying squirrel population genetics

Glaucomys sabrinus population genetics

3. **range of dates searched:**

Dates were not restricted to a range

4. **other resources used:**

Wilson, D.E., Lacher, T.E., Jr & Mittermeier, R.A. eds. (2016). Handbook of the Mammals of the World. Vol. 6. Lagomorphs and Rodents I. Lynx Edicions, Barcelona.

9. Will any of the animals described in this protocol be housed in an animal facility?

☐ Yes ☒ No

If yes, check the appropriate facility below:

- ☐ Biological Sciences Animal Rooms
- ☐ Fish Hatchery
- ☐ Samoa Aquaponics
- ☐ Telonicher Marine Lab
- ☐ Wildlife Pens
- ☐ Zebra Fish Development Lab
- ☐ Other. Please list: Click or tap here to enter text.

9a. Facility managers must be consulted prior to submitting protocol form. Please enter the date the manager was consulted: Click or tap here to enter text.

10. Scientific name, common name, and characteristics of all species to be used. List species separately to explain variation in use. Please also list the total numbers of animals to be used or substantially affected by this project.

For field studies, please list all target species and note their status (not protected = NP; protected, including species of special concern or candidate species = P; considered by the state or federal government to be threatened = T, considered by the state or federal government to be endangered = E); also list non-target species that are likely to be impacted. List the range of numbers of individuals to be used for each species.

TARGET SPECIES - please attach additional pages if needed

Latin Binomial(s)	Common name(s)	Sex	Age or Wt Range	Status	Numbers
Glaucomys sabrinus/oregonensis	Humboldt flying squirrel		Unrestricted		30
Glaucomys sabrinus californicus	San Bernardino flying squirrel		Unrestricted		30
Click or tap here to enter text.	Click or tap here to enter text.		Click or tap here to enter text.		Click or tap here to enter text.
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NON-TARGET SPECIES – please attach additional pages if needed

Latin Binomial(s)	Common name(s)	Status	Numbers
Tamiasciurus douglasii	Douglas squirrel		Variable, but much less than 30
Sciurus griseus	Western gray squirrel		Variable, but much less than 30
Eutamias sp.	Chipmunks		Variable, but much less than 30
Mustela erminea	Ermine		Variable, but much less than 30

- 11. Explain why a smaller number would not allow you to meet your objectives (please provide justification based on statistical or other logical reasoning). If this is a field project, and you cannot predict the exact number of animals to be sampled, please give your best estimate and an explanation of the variables that will determine your sample size. N/A is an inappropriate response unless the protocol covers only the transportation, use, and/or storage of carcasses or tissues.**

Thirty samples are the minimum number needed for a robust genetics study at the population level that is statistically sound (Hale et al). Although that is the goal, in reality we may capture far fewer individuals. The actual number captured may vary due to factors such as: weather tripping or damaging the trap, other non-target species triggering the trap instead, the traps may not successfully collect hair samples, and the flying squirrels may avoid the traps.

Literature Cited: Hale ML, Burg TM, Steeves TE (2012) Sampling for Microsatellite-Based Population Genetic Studies: 25 to 30 Individuals per Population Is Enough to Accurately Estimate Allele Frequencies. PLoS ONE 7(9): e45170. doi:10.1371/journal.pone.0045170

- 12. Source of the animals (or tissues) to be used for captive studies or the location of study area(s) for field studies. For transportation, storage, and use of tissues from carcasses, explain the circumstances of death. If this information is unknown, provide the name and contact information for the person or company from which the samples are to be obtained.**

The field sites will be the Headwaters Forest Reserve, land managed by Green Diamond Resource Company in Humboldt County, private land in Humboldt, Kern Los Angeles and San Bernardino Counties, and the San Bernardino National Forest.

- 13. Will live vertebrate animals be maintained in captivity for greater than 12 hours?**
☐ Yes ☒ No

If yes, describe where and how the animals will be housed (include all relevant husbandry details):

Click or tap here to enter text.

Who will be responsible for their daily care? Click or tap here to enter text.

14. List the specific procedures likely to affect the behavior, physiology or wellbeing of live animals.

Modified Tomahawk hair snare traps, modeled after the ones described in Trapp and Flaherty (2017), will be used to collect hair from flying squirrels. Essentially a wire brush is attached to the door of the tomahawk trap, while the metal tab which normally prevents the trap from opening will be wired to the door to allow the animal to push its way out of the door after the trap has been triggered. While the animals will be startled upon the trap triggering, they should not incur any injuries, and seconds to minutes after the trap has been set the animal can push the entry door open and (ideally) deposit a hair sample on the attached wire brushes.

Camera traps will be set up facing the hair snares to double check that any hair collected is from a target species.

15. Mark the level of expected pain or distress caused by your methods below.

- ☒ The methods described are purely observational and non-invasive OR will involve only the tissues or carcasses of dead animals; behavior of live animals will not be influenced intentionally.
- ☐ The methods will affect behavior, but no animals will be captured or handled (e.g. baiting animals, cameras in close proximity to animals, production of noises within normal limits of volume and frequency)
- ☐ The methods involve capture or handling without anesthesia, but only for a brief period for measurement or observation. No samples will be collected.
- ☒ The methods involve capture or handling without anesthesia, and routine samples (hair, blood, etc.) will be collected or euthanasia will be performed; this may involve use of routine pharmaceuticals to promote health (e.g. antibiotics, vitamins, fluids). This work may also involve temporary marking, placement of permanent tags, or fitting with telemetry transmitters or GPS receivers.
- ☐ The methods require use of anesthesia to mitigate distress or facilitate handling, and routine samples (hair, blood, etc.) will be collected or euthanasia will be performed. As above, this work may involve temporary marking, placement of permanent tags, or fitting with telemetry transmitters or GPS receivers.
- ☐ The methods require use of anesthesia to mitigate pain or distress, and procedures will be invasive enough to require pain killing drugs (analgesics) upon revival. Sampling and marking may be performed as above.
- ☐ The methods will cause pain or considerable distress, but analgesics will not be used to mitigate the pain (e.g. surgeries from which animals are revived without provision of analgesics).
- ☐ The methods will be invasive and cause prolonged physiological or psychological stress without adequate mitigation of pain or distress. This may involve allowing animals to progress to death without provision of euthanasia or analgesia (e.g. LD50 experiments or long-term food or water deprivation).

16. Provide a complete and detailed description of all procedures to be performed involving live

vertebrate animals. This response should justify comments made in # 13-15 and provide a detailed explanation of all procedures that affect animal behavior, physiology or wellbeing. Your response must address the handling and restraint of animals; deprivation of food or water; use of chemicals or biological agents; sampling methods for removal of biological samples; surgical and post-surgical procedures. N/A is an inappropriate response unless the protocol covers only the transportation, use, and/or storage of carcasses or tissues.

Following the study conducted by Trapp and Flaherty (2017), we modified Tomahawk traps so that they would become single-use hair snares that would not capture and hold an animal. The locking mechanisms on the doors were disabled by tying metal wires and zip ties around it, so that the flying squirrels will be able to enter, eat the bait, and push on the door to leave. No direct handling of individuals or permanent capturing will be necessary. Wire gun cleaning brushes were attached to the bottom edge of the door so that when the flying squirrels exit, hair samples will be deposited on the brushes. A strip of fly trap paper was also attached to the inside of the Tomahawk trap right before the bait, in front of the trigger flap, as an attempt to get more hair samples. Pictures of the modified hair snare are attached as a separate file. The bait being used is a standard suet cake of nuts and seeds and is placed toward the back of the trap. Traps will be set about 2 meters above ground on trees with known flying squirrel presence, or on the ground. Remote cameras will also be attached to trees facing the trap. These cameras will take pictures when motion is detected, and the pictures will be used to check if flying squirrels are entering the hair snares.

In the study, 316 squirrels (of 3 different species) successfully entered and exited the modified Tomahawk trap, but there was 1 death of a non-target species. That individual chewed the zip ties that stopped the locking mechanism, and thus became trapped and unable to leave. In addition to zip ties, we used metal wires as a fail-safe to reduce the probability of an animal chewing through zip ties as was done in the published study.

Ten traps will be set out each day, over the course of 3 days, in the San Bernardino National Forest and in private land in San Bernardino County, until a total of 30 samples are obtained from the San Bernardino region. Ten traps will also be set out each day, over the course of 3 days, at the Humboldt County field sites, until a total of 30 samples are collected within Humboldt County. As the traps are modified to prevent prolonged entrapment of the animal (as the animals can push the door open following door closure), we will check traps once a week.

Literature cited: Trapp SE, Flaherty EA. 2017. Noninvasive and cost-effective trapping method for monitoring sensitive mammal populations. *Wildlife Society Bulletin*. 41(4):770–775. doi:10.1002/wsb.824.

17. Use of animals for teaching or research requires consideration of alternative procedures to reduce the number of animals used and the pain and suffering caused by animal use. Explain how you determined whether alternative procedures were feasible for your study.

Please refer to the Altweb website (<http://altweb.jhsph.edu/resources/searchalt/>), which provides links to search engines and provides general information on alternatives, for help in searching for alternatives to animal use.

1. the type of literature searches conducted:

Altweb

2. keywords used:

Flying squirrel noninvasive hair snares

Squirrel noninvasive hair snares

Flying squirrel noninvasive survey

Flying squirrel hair sampling

- 3. range of dates searched:**
1980 - present

- 4. other resources/methods used to determine alternative procedures:**

Long, R.A., MacKay, P., Zielinski, W.J., & Ray, J.C. eds. (2008). Noninvasive Survey Methods for Carnivores. Island Press.

- 18. Describe alternative procedures that were considered and rejected and a brief explanation of why the alternative procedures were rejected. N/A is an inappropriate response unless the protocol covers only the transportation, use, and/or storage of carcasses or tissues.**

This method of hair sampling is the alternative to sampling from individuals by capturing and directly handling them. One other idea was to attach double-sided tape and wire gun cleaning brushes inside a short PVC pipe. Bait would also be placed inside, and the flying squirrels would go into the pipe, eat the bait, and deposit hair samples on their way out. This idea was rejected because in a previous project led by the US Fish and Wildlife Service, these hair snares did not successfully collect hair when placed in the San Bernardino National Forest. And because the pipe is open on both sides, there is a higher likelihood of contamination and collecting hair from non-target species.

- 19. Identify serious human health risks (non-routine exposures to risk, disease agents, toxic chemicals, dangerous environmental conditions, etc.,) to which any participants might be exposed during the routine performance of the duties proposed herein.**

The habitat of the Humboldt and San Bernardino flying squirrels is montane coniferous and hardwood forests. Certain areas may be rugged, steep, and/or have dense understory.

Describe steps taken to mitigate risks.

Proper field work clothing will be worn such as hiking boots, and we will obtain information about each site before setting up hair snares to plan the easiest routes in and out of an area. No one will set up traps or collect them alone, and we will work during daylight hours. In addition, we will carry safety equipment such as flashlights, phones, pocket knives, a first-aid kit, and drinking water. Many of the sites will also be private property, and as appropriate we will consult with land owners about risks and mitigations steps. Many traps will be set in backyards at bird feeders the flying squirrels are known to inhabit, and those sites have relatively few risks involved.

- 20. Describe the fate of the animals upon completion of the protocol. Include (1) the procedure for euthanasia whether necessary as an experimental termination or in the case of unanticipated, accidental, injury whenever animals will be confined or handled and (2) the method of verification of death. Chemical euthanasia methods must include an appropriate, pharmaceutical-grade, drug, the route, and the dose to be used. Applicants should review the current Guidelines for Euthanasia (or its replacement in the Code of Federal Regulations), and justify any variations from the approved methods. Note that the Responsible Faculty Member must report unexpected deaths to the IACUC immediately and that N/A is an inappropriate response unless the protocol covers only the transportation, use, and/or storage of carcasses or tissues.**

It is expected that the individuals will still be alive after entering the trap, and will be able to leave by pushing on the door of the hair snares. Should a trap mortality occur isoflourane will be used to euthanize any mortally injured animals by placing a saturated cotton ball in a plastic bag with the animal. However,

this is extremely unlikely due to the hair snare design.

21. I certify by checking each of the boxes below, that all of the following are true:

- ☒ I have read and agree to abide by the "Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training at HSU," and that I will make copies of these principles and other pertinent guidelines available to those persons who work under my supervision, and that deviations from this protocol, including any unanticipated injuries or death of animals, will be reported immediately to the IACUC.
- ☒ My level of supervision will be such that the procedures outlined in this protocol will be carried out in a humane and a scientifically acceptable manner as described herein.
- ☒ I take responsibility for the conduct of anyone working under this approved protocol, and I will supervise the research to ensure that no work is conducted that is not covered herein or in a separate approved protocol.
- ☒ I will ensure that no work described in this protocol will begin until the protocol has been fully approved by the IACUC, and that I will adhere to all deadlines and procedure outlined in the HSU ANIMAL WELFARE ASSURANCE in accordance with the PHS Policy for Humane Care and Use of Laboratory Animals.
- ☒ I am aware that my research might require permits from federal and/or state agencies that regulate the harassment, capture, transport, captive maintenance, handling and manipulation of live vertebrate animals.
- ☒ My research will be conducted in accordance with all relevant federal and state laws.
- ☒ My study does not unnecessarily duplicate previous studies using live vertebrate animals, as determined through literature database searches.
- ☒ I have considered the use of less invasive procedures, use of fewer numbers of animals and have determined that the methods proposed in this protocol are justified for the research and/or instructional objectives described herein.

I am aware that the following Acts apply to my study (check all that may apply):

- ☒ Animal Welfare Act
 - ☒ State of California Fish and Game Commission (Title 14) - Scientific Collecting Permit(s)
- ☐ Endangered Species Act
- ☐ Fishery Conservation and Management Act
- ☐ Lacey Act
- ☐ Marine Mammal Protection Act
- ☐ Convention on International Trade in Endangered Species of Wild Fauna and Flora
- ☐ Other: please list Click or tap here to enter text.


Signature, Responsible Faculty Member

11/29/2018
Date