

NOV 28 2016

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: 3/11/16 Revision 1 Date: 11/7/16 Revision 2 Date: 11/28/16 No. 15/16.W.96 - 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 euthanatized 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

Signature, IACUC Member

Date

☒ Approved

() Denied

Signature, IACUC Member

Date

☒ Approved

() Denied

Signature, Campus Veterinarian (if necessary) Date

() Approved

() Denied

Signature, IACUC Chair

Date

☒ Approved

() Denied

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

Protocol Edition 3/16/2016

HUMBOLDT STATE UNIVERSITY
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INSTRUCTIONS

Federal animal welfare regulations require that an Institutional Animal Care and Use Committee (IACUC) review and approve all activities involving the use of vertebrate animals prior to their initiation. This includes any animals used for the development of experimental methodologies, instructional purposes, research, etc. Approved protocols for ongoing and recurrent activities must be reviewed by the IACUC on an annual basis. However, extensions and amendments requiring an abbreviated application process may be granted for a total of three consecutive years. Compliance with animal welfare regulations is mandatory and is the responsibility of all individuals (including faculty and students) who choose to work with live vertebrate animals.

To avoid the proliferation of submissions, please provide generic descriptions (including multiple routes of compound administrations, minor procedural variations, similar laboratory exercises from a single course, routine exercises used in several courses, etc). When multiple vertebrate species are to be used, please clearly describe all procedures, and all variations thereof, to be used with each individual species.

Please submit your protocols to the Dean's Office, College of Natural Resources and Sciences, Forestry Bldg, Room 101. All protocols should be submitted on the most recent version of the forms downloaded from the IACUC web page (<http://www.humboldt.edu/iacuc>). You can expedite the review process by following these formatting rules: leave an extra blank line between the questions and your responses; leave questions in bold-face type; type your answers in regular (non-bold) type; and do not delete anything from the questions. Please contact the Campus Veterinarian, Dr. Rick Brown, (by phone, 826-3320, or e-mail, RBrown@humboldt.edu) or the Chair of the IACUC, Dr. Rick Zechman (by phone, 826-3546, or by email Rick.Zechman@humboldt.edu) with questions concerning protocol preparation and submission.

- ♦ Please allow ten working days for review of proposals to conduct minimally invasive procedures and an excess of one month for review of proposals to conduct invasive procedures; note that these time periods are minimal and assume that no revisions will be necessary prior to approval. ALWAYS verify approval (Office of the Chair of the IACUC; 826-3256) before starting your project. Authors of protocols should contact the Campus Veterinarian, the Chair of the IACUC or Violet McCrigler in the CNRS Dean's Office, if they haven't heard any news after 10 days following protocol submission.

1. Faculty Project Leader: Micaela Szykman Gunther

Department Affiliation: Wildlife

2. Project Title: Roosevelt elk capture, health assessments, and monitoring in Humboldt and Del Norte Counties

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

Micaela Szykman Gunther <micaela.szykman.gunther@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 list students in a class here - see 8 below):

- Rick Brown – faculty in wildlife and wildlife veterinarian; has decades of experience with capture, handling and monitoring of small, medium and large mammals. He has worked on captures of Roosevelt elk, Tule elk, three subspecies of mule deer, bighorn sheep, carnivores ranging in size from spotted skunks to black bears, a variety of rodents, lagomorphs, etc.
- Tim Bean – faculty in wildlife; has considerable experience with capture and monitoring of rodents, including kangaroo rats and porcupines, as well as a current project with pronghorn antelope.
- Carrington Hilson – MS in wildlife from HSU; has experience working on and leading captures of large mammals in both Colorado and Arizona. Carrington is currently working as an environmental scientist coordinating the elk studies for CDFW in Humboldt and Del Norte counties. She'll lead on captures, conduct necropsies, and coordinate all field work on the project.
- David Lancaster – MS in wildlife from OSU; working with CDFW in Eureka; has a couple of decades of experience with capture and processing a variety of wildlife species, including deer and elk.
- Richard Callas – MS in wildlife from HSU; senior biologist for the northern region of CDFW; has decades of experience capturing and handling a variety of medium and large size mammals.
- Erin Nigon – MS student in wildlife at HSU; has worked as a capture team member and leader on wildlife captures in AZ and as a team member in Colorado (her experience includes large ungulates); she will also be involved in necropsies and sampling. She won't be darting, but she'll be involved in all other aspects of the work.
- Emily Buck – MS student in wildlife at HSU; will help on captures and monitoring of elk. She won't be darting or working independently on captures, but she'll help on all aspects. Her thesis will depend on collection of samples from elk.
- Rudy Mena – MS student in wildlife at HSU; will help on captures and monitoring of elk. He won't be darting or working independently on captures, but he'll help on many aspects. His thesis will depend on collection of fecal samples from elk as well as behavioral observation on elk.
- Ben Gonzales is a senior veterinarian working at the Wildlife Investigations Lab for CDFW. Ben will oversee all aspects of the captures.
- Several undergraduates may assist on elk captures but will always be under direct supervision. We can provide names of these students when we have capture teams formed, but these may include Kara Cundall, Emily Aarsvold, Bayan Ahmed, Alex Benn, Holly Gamblin, Mackenzie Spencer, Alessandra Phelan-Roberts, Wade Burris, Jessica Whalen, Gina Culver and Bear Culver. These are all senior students in the Wildlife program who have spent months observing elk in the region.

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:

Funding for this project is split between our research group at HSU funded by a CDFW contract to Bean, Brown and Gunther (administered by HSUF) and internal CDFW funding for elk research and monitoring to Hilson (these are CDFW monies administered by and for the agency – but in collaboration with HSU researchers).

- 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.

Date of Approval.

- 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 study will provide information needed to manage and conserve elk in NW California by examining abundance, distribution, population demographics, space use, migration corridors, migratory behavior, and health status. Comprehensive and coordinated assessments of their population status and ecology are needed to provide for the continued recreational and economic benefits of these species and provide for their conservation for the future. Although many studies have been conducted using different monitoring methods for elk, the most commonly used methods involve aerial monitoring which doesn't work in the dense forests of our region. This project is necessary to ensure the CDFW is able to make sound management decisions in regards to the conservation and restoration of these highly valued animals in California.

- Our methods will include capture of elk cows, a few bulls, and 30+ recently-borne calves by darting or with corral traps of cows and bulls; calves will be hand captured as soon after birth as possible. Cows and bulls will be fitted with GPS collars, regular VHF radio-collars, identification collars and ear tags. Calves will receive numbered ear tags, and some will receive ear tag VHF transmitters. GPS collars and VHF radios will allow detailed analysis of distribution, movements, herd constitution, interactions, and behaviors. We will also assess calf survival and recruitment as well as conduct necropsies on all adults and calves that die during the study. We will collect data and samples related to health during captures and by collecting fecal samples from known individuals during the following year to allow the potential for evaluation of pathogen transfer from wild elk to domestic cattle and vice versa. Elk will be captured during each of the next two seasons (Nov-March of 2016/17 and 2017/18); we'll be monitoring locations, habitat use and behaviors during each of the next three years. We understand that annual renewal of IACUC approval will be necessary for the continuation of the project beyond the first year. .

- 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): Undergraduate and Graduate students doing independent projects, WLDF 490, WLDF 495, WLDF 695.

CRN: CRNs for these courses vary with instructors – all who handle elk will need to submit the health assurance form.

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.

Listed in answer to Q4.

Describe the learning objectives that merit using live animals for the purposes of instruction.

The main purpose of the study is research, but we'll take every advantage to involve undergrads and graduate students in as many aspects and side projects as possible. None of the work is being conducted primarily for education.

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:

We used a variety of search engines, including BioOne, Wildlife Ecology Worldwide, Agricola, Promed, Google Scholar, etc. The problem is that no-one knows how best to monitor elk in our region, so we did not consider not capturing and not using radio/GPS collars to assess different monitoring strategies. However, these literature searches were used to consider different anesthetics and a variety of minimally invasive methods for monitoring elk.

2. keywords used:

Elk, Cervus canadensis, Cervus elaphus, demographics, movements, behavior, cow-calf, habitat use, health, disease, parasites, and other keywords more specific to behaviors, landscape ecology, habitat use, health, disease, and interactions with cattle.

3. range of dates searched:

Dates were not constrained

4. other resources used:

Books on wildlife management, mammal management, population ecology, wildlife diseases, depredation by elk, etc. Many conversations with biologists and administrators working with CDFW or in academia.

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.

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
<i>Cervus canadensis roosevelti</i>	Roosevelt elk	Unspecified	Adult cows, bulls	NP	60 cows and 5-10 bulls will be captured and monitored this year to be repeated in our second year of the study
<i>Cervus canadensis roosevelti</i>	Roosevelt elk	Unspecified	Calves	NP	30-60 calves will be captured and monitored this year to be repeated in our second yr of the study.

<i>Cervus canadensis roosevelti</i>	Roosevelt elk	Select One	All	NP	400-500 elk will be observed during each year of the study
Click or tap here to enter text.	Click or tap here to enter text.	Select One	Click or tap here to enter text.	Select One	Click or tap here to enter text.
Click or tap here to enter text.	Click or tap here to enter text.	Select One	Click or tap here to enter text.	Select One	Click or tap here to enter text.
Click or tap here to enter text.	Click or tap here to enter text.	Select One	Click or tap here to enter text.	Select One	Click or tap here to enter text.

NON-TARGET SPECIES – please attach additional pages if needed

Latin Binomial(s)	Common name(s)	Status	Numbers
Click or tap here to enter text.	Click or tap here to enter text.	Select One	Click or tap here to enter text.
Click or tap here to enter text.	Click or tap here to enter text.	Select One	Click or tap here to enter text.
Click or tap here to enter text.	Click or tap here to enter text.	Select One	Click or tap here to enter text.
Click or tap here to enter text.	Click or tap here to enter text.	Select One	Click or tap here to enter text.

- 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.**

Elk will be captured and studied in three main areas in Del Norte, Northern Humboldt, and Central Humboldt counties. Apparent herd (or group) sizes fluctuate, but it is unknown how much groups move among and between areas and how often groups split up and form new groups. We have chosen to

capture and collar approximately 12 elk from each of the three main areas listed in the answer to Question 12 (but that are found in different subgroups) in the first year, and possibly another 30-40 elk the second year of the study. These numbers are considered the minimum necessary to capture enough intragroup movements and fragmentation of groups to represent elk throughout the region. Elk calves will be monitored to evaluate survival and cause-specific mortality. Evaluation of survival requires monitoring of enough animals that it is likely that some will die from natural causes. Although we would like to monitor more calves to get a more accurate assessment of cause specific mortality, captures and monitoring are very labor intensive and it is doubtful that we could monitor more than 60 calves even if we had the money and crew members to capture more calves (which we don't).

- 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.**

Wild and free-ranging elk will be captured in three main areas: (1) Coastal Del Norte county from north of the Smith River to the Mill Creek drainage, (2) northern Humboldt County, including Bald Hills Road, Gold Bluff Beach, Prairie Creek Redwoods State Park, Redwood National Park, Stover Ranch, Davison Ranch, Orick, Stone Lagoon & Big Lagoon, and (3) central Humboldt County.

- 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.**

Baiting for corral traps; darting and anesthesia; placement of collars and ear tags, hand capture of calves will stress mothers and calves, monitoring of VHF radio signals, and observation from a reasonable distance (which is intended not to affect the elk, but it might sometimes).

- 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.

- This is a large study in which HSU researchers and students will be collaborating with the California Department of Fish and Wildlife biologists. Elk will be studied to evaluate herd size and composition, movements, habitat use, behavior, demographics, survival and recruitment, cause-specific mortality, population health and disease. We plan to observe a total of up to 500 elk existing in many different locations on multiple occasions throughout the year. We also plan to capture and place collars with VHF radio-transmitters and GPS receivers on 32 cows and 3 bulls and VHF ear tags on 30 calves during each of the next two years (these numbers will be reconciled with numbers in my response to #10 below). Routine monitoring will include observation of as many elk as often as is possible, monitoring of VHF signals to determine whether animals are alive or dead as well as to evaluate movements of the head and neck, retrieval of data from GPS collars to determine home range use, habitat use and movements.

- Adult elk will be captured opportunistically via chemical immobilization from the date of IACUC approval through March, 2017 (and then yearly if amendments are approved). This will be accomplished using free-range darting and through use of corral traps. Individuals caught in a corral trap will be immobilized and processed in the same manner as individuals obtained through free-range darting. Elk will only be captured in locations and at times where authorized hunting is not taking place; no elk will be captured 45 days prior to, or during, any recreational hunting season (which vary among different properties and public lands). Cow elk will only be captured during the first and second trimester of pregnancy to avoid potential adverse effects to the developing fetus that can occur in the third trimester. Capture of bull elk is also limited to

the time of year when antlers are fully developed, have been shed, or when antlers in velvet are still short to avoid damage to vascular tissues and subsequent deformations of fully developed antlers.

- Individual capture crews will dart only one elk at a time. Free roaming elk may be approached slowly on foot (to within 10-50 m) or in vehicles along dirt roadways. Attempts to dart additional animals will only occur when any previously immobilized elk has been fully processed and successfully released. When multiple individuals have been captured in a corral trap (see below) a short range projector (pistol or air-driven rifle) will be used to chemically immobilize target individuals. A capture crew consisting of at least 4 people will be onsite for processing. All elk will be immobilized and antagonized by personnel certified in chemical immobilization by the CDFW Wildlife Investigations Lab (WIL – including CDFW biologists and R. Brown). Darting will be conducted using either the Pneu-Dart (CDFW) or Dan Inject projection systems (R Brown) and sterile, disposable, 2 or 3 cc Pneu-Dart darts; thorough searches will be conducted for any darts which may miss their targets.

- Hexagonal corral (or panel) traps may be used for an unknown number of captures. These corrals will be constructed of six portable, steel tube, livestock panels measuring 2.74 m (high) x 3.65 m (length) and be approximately 8 m in diameter. Plywood sheets (1.2x2.4 m) will be attached to the top of the corral to extend the height to 3.65 m and to block visual stimulation. The entrance will be approximately 1.02 m wide with a steel tube swing door reinforced with plywood. The door will have a spring loaded hinge, opening to the inside that will shut when animals push past a strong fishing line hung in front of the bait (fresh alfalfa). This line will track around the outside of the trap to a post near the open door. At the top of the post, the fishing line will connect to a rat-sized snap trap that will be used to release the door and let it swing shut. VHF radio transmitters will be attached to the gate mechanism to allow prompt response when the elk become captured. Wooden posts will be set at the doors and the back of the trap to stabilize the door and sides. No chutes will be used to constrain animal movements.

- Free-roaming or corralled elk will be immobilized using BAM: a combination of butorphanol tartrate, azaperone tartrate, and medetomidine hydrochloride at a dosage of 0.17 mg/kg, 0.06 mg/kg, and 0.07 mg/kg, respectively. BAM is a fairly new drug combination available through ZooPharm in 11 ml vials; each ml contains 27.3 mg of butorphanol tartrate, 9.1 mg azaperone tartrate, and 10.9 mg medetomidine HCl. Cow elk (270 kg) will be darted with 1.7 mL of BAM in 2-cc darts and bull elk (400 kg) will be darted with 2.6 mL of BAM in 3-cc darts administered IM. If anesthesia is not achieved within 20 minutes, up to a full dose of BAM may be re-administered IM. If adequate anesthesia is not achieved on a consistent basis the initial dose of BAM will be increased to 0.20 mg/kg butorphanol, 0.07 mg/kg azaperone, and 0.08 mg/kg medetomidine after consultation with Ben Gonzalez, Senior Veterinarian at the Wildlife Investigations Lab of CDFW. BAM is a fairly new drug combination being used on wildlife, and I can provide references if necessary.

- All elk will be handled, restrained, and monitored to prevent injury to them as well as the capture personnel. During processing, handlers will record information specific to each animal on capture data sheets. Elk will be kept in a sternal or left lateral position and hobbles will be attached to front and rear legs during processing. Ophthalmic ointment and eye covers will be placed on immobilized elk to prevent damage to their eyes and to limit visual stimulation of the anesthetized elk. Vital signs including body temperature, respiration rate, and heart rate will be monitored at 5 minute intervals. Water, or isopropyl alcohol in remote areas, and blankets will be available to help regulate body temperature, if needed. Body temperature will be considered normal between 99 °F and 104°F. If body temperature falls outside this range immediate action

will be taken to bring body temperature back into normal range. If cooling or warming actions are taken and the animal becomes progressively more hypothermic or hyperthermic, anesthesia will be immediately reversed. BAM has been associated with decreased oxygen levels in tissues (hypoxia), so supplemental oxygen will be supplied at a rate of 2 liters per minute throughout the period of anesthesia. If respiratory depression becomes apparent the Jen Chung acupuncture point will be used to increase the respiratory rate. This involves inserting a hypodermic needle (20 gauge, 1.5 inch) into the center of the nose, ventral to the nostrils and rotating the needle. If respiration rate does not increase, then doxapram will be administered IV (slowly) at a dose of 1 mg/kg. If the animal becomes increasingly hypoxic and the respiration rate remains less than 4 breaths per minute, then anesthesia will be immediately reversed.

- Processing of the animal will occur at the capture location. The capture crew will collect up to 20 cc of whole blood by jugular venipuncture (only by experienced personnel). Blood will be stored in serum tubes and EDTA tubes which will block the formation of clots. Blood will be assayed for serum antibodies and tested for pregnancy. Ectoparasites and fecal pellets will also be collected. Age will be estimated by tooth wear and body condition will be scored on a range of 1-6. Herd health will be surveyed by screening biological samples for exposure to diseases and parasites loads. Samples will be processed per the methods and locations identified by the Wildlife Investigations Laboratory (WIL). Pregnancy will be determined by a serum assay for pregnancy-specific protein B (Stephenson et al. 1995) and by ultrasound on site. Additionally, age and body condition will be scored on a range of 1-6 following Cook (2010). Triple antibiotic ointment will be applied to location of skin biopsies and where abrasions or lacerations are observed due to capture.

- Each captured elk will be given quick physical exam to evaluate injuries or obvious abnormalities. Wounds caused by the dart will be treated with Dry-Clox (cloxacillin benzathine). All darted elk will receive a systemic antibiotic, Combi-Pen 48 (penicillin G benzathine and penicillin G procaine; 300,000 units/mL), administered subcutaneously. Only 10 ml of antibiotic will be injected per site and the needle will be redirected after 5 ml to avoid pain and tissue damage. Cow elk will receive 20 mL Combi-Pen 48 and bulls will receive 29.5 mL Combi-Pen 48.

- Plastic cattle ear-tags (with visible alpha-numeric codes), including those with radio transmitters for calves (these tags are being purchased by CDFW and are currently out to bid) or marking collars (simple 8 cm wide leather collars with alpha numeric codes to allow ID of individuals) for adults that do not get GPS/VHF collars will be affixed to each captured individual. A 4-mm punch biopsy will be used to create a hole in the center of the ear with care to avoid visible blood vessels. The punch biopsy will be placed into a vial with desiccant to preserve the DNA sample. The ear tag will then be attached through the existing hole using applicator pliers designed by the tag manufacturers. Collar(s) will be affixed to capture animals with care to ensure that collars are fit snugly and do not slide up and down the animal's neck. The collar should not be so loose as to allow the animal to fit a hoof through it or for the collar to slide excessively along the length of the neck. The collar also should not be so tight as to rub up against the neck, which can cause hair loss and damage the skin. The collars (Lotek Iridium Track M 2D satellite collars with time released drop-off weigh a total of 835 g) used for bulls and cows; bull elk will have a magnetic expansion in the collar to accommodate changes in neck circumference that normally occur during rut. Pregnancy of captured females will be determined using a portable ultrasound. Vaginal implant transmitters (VITs) will be used to notify us when a calf is borne. VITs (3.5" x 0.64" with 2.25" wings and weighing 26.3 g) will be placed using sterile, disposable, equine speculums from MWI Veterinary Supply. VIT

signals will be paired with the Iridium GPS collars worn by the female and will transmit a signal upon expulsion of the transmitter. After handling is complete, the anesthesia will be chemically reversed using atipamezole and naltrexone at dosages of 0.324 mg/kg and 0.009 mg/kg IM, respectively, and the elk will be released at the site of capture. Processing of animals will be done as efficiently as possible to minimize time under chemical anesthesia. As animals awaken from anesthesia, they will be observed for as long as possible (until wander out of sight) to maximize safety of animals that have just been released.

- The VIT implants will signal us when a calf is born, and we'll radio-track it to its location. Elk calves remain hidden and do not join the herd until they are 10-14 days of age. During this time, it is possible to slowly walk in on calves and capture them (carefully yet firmly) by hand. At least two people will conduct all calf captures to ensure safe captures and to provide extra vigilance for the return of the cow. Elk calves will be handled carefully without anesthesia. Hobbles and a quick-release eye guard will be used during the restraint. Measurements will be taken and ear tags will be placed as described for adult elk. Sampling will be similar to adults. Calves will be released at the site of capture as soon as the sampling and tagging procedures are completed.

- Triple antibiotic ointment will be applied to the location of ear-tags and where abrasions are observed. Wounds caused by the dart will be treated with DryClox (cloxacillin benzathine). All darted elk will receive the systemic antibiotic Combi-Pen 48 (combined penicillin G benzathine and penicillin G procaine; 300,000 units/mL), administered subcutaneously. Only 10 ml of antibiotic will be injected per site and the needle will be redirected after 5 ml to avoid pain and tissue damage. The "average" cow elk will receive 20 mL of Combi-Pen 48 and the average bull elk will receive 29.5 mL Combi-Pen 48. Again, all elk will be released at the site of capture following reversal of anesthesia (cows and bulls), attachment of collars and ear tags, and once sampling is complete. All elk will be observed after release for a minimum of 5 minutes or until they run out of our field of view (as typically happens quickly).

- Locations of elk cows and bulls with collars will be recorded at programmable intervals (perhaps hourly during daytime and every couple of hours during the middle of the night), but this has yet to be decided precisely. VHF collars will be used to locate individual animals and their groups to facilitate behavioral studies. In addition, VHF transmitters transmit a mortality signal when the animal has stopped moving during an entire 24-hour period.

- Observers will monitor elk from a distance that avoids disturbance of elk behavior. Such distances will be typically be in the range of 50 to 200 m, but may be less for elk that graze along roadways or in accessible pastures. Elk will not be disturbed intentionally by these observations.

- The Lotek collars that we're using will drop off automatically after approximately 156 weeks of service (and retrieved by locating the associated VHF transmitter), so elk will not need to be re-anesthetized to remove the collars. Ear-tags will remain in place as a permanent identifier for the life of the elk.

- 17. Use of animals for teaching or research requires consideration of alternative procedures to reduce the number of animals used and to decrease the pain and suffering caused by animal use. Describe alternative procedures that were considered and rejected as well as 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.**

We have evaluated literature concerning all aspects of this study. Unfortunately, the contemporary methods used to monitor elk populations are not considered effective in the forests of NW CA, and there is no literature that allows us to effectively extrapolate or model monitoring effectiveness. We have used the websites at <https://www.nal.usda.gov/awic/alternatives> and at <http://guides.lib.ucdavis.edu/content.php?pid=669778&sid=5546194>. These sites provide useful references, but nothing published explains how to evaluate monitoring techniques without actually monitoring the animals as confirmation of the methods used. We believe that our methods are “minimally” invasive, requiring only the placement of radio and GPS collars to follow elk. In short, we’re doing everything we know of to minimize impacts on individual elk.

- Alternatives? We have considered not using collars, but finding individual elk, and monitoring movements of elk, is very difficult without the use of radio transmitters. We considered using only VHF collars to minimize weight associated with the GPS collars, but the weight of the collars is minimal in comparison with the weight of the elk and the data obtained from GPS receivers will be orders of magnitude more helpful than would be VHF collars alone. Although we have discussed following individual animals based on scars or antler formations, the ability to identify individuals using such methods is extremely limited. We certainly considered different drugs to immobilize the elk. We will use a fairly new anesthetic combination called BAM. This drug combination comes with less risks to handlers, and less risk associated with lost darts, than does carfentanil (a narcotic often used for chemical immobilization of large mammals). Since we’re collaborating with CDFW, their employees need to be able to use the drugs in the field, and carfentanil is only appropriately used by veterinarians trained both in its use and in the rescue of someone that might be inadvertently exposed: most wildlife biologists lack such training. BAM is also smoother and more consistent than the more traditional choices involving ketamine, xylazine or dexmedetomidine. Telazol is always an option for immobilization, but the recovery period can be long and there is no good reversal agent; leaving recovering animals somewhat at risk.

18. 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.

In order of decreasing risk... the greatest risks to our crew will occur while we are capturing and sampling elk calves; elk cows can be dangerous. The BAM used as the anesthetic might also be dangerous if injected accidentally into a person. Elk might guard other elk that are immobilized (as in bulls defending immobilized cows during the rut), but this is rarely a serious risk when capturing animals outside the normal rut period.

Describe steps taken to mitigate risks.

People capturing elk calves will work in teams, and people capturing adults will work in crews with five to six people. Cows that appear moderately aggressive will be chased away or at least not allowed to attack the handler. If a cow becomes unmanageably aggressive, then the calf will be released and the capture crew will quietly and quickly move away from the calf. All darters using BAM will be fully educated about the risks, and the reversal agents, naltrexone and

atipamezole, will be available at all times.

19. 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.

If an elk is found injured, or becomes injured during the processing, the suffering will be stopped with a gunshot to the braincase. Shooters, or wardens, from CDFW will be available at all times (as facilitated through Carrington Hilson, CDFW, and HSU faculty, staff and students will not be involved in shooting. If an elk is shot by CDFW, death will be verified by a lack of reflexes, breathing and heart beat either by visual assessment for 5 minutes or by auscultation of the chest with a stethoscope at intervals until 5 min after estimated time of death.

20. I certify that the above information is accurate and complete, that 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," 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. Further, my level of supervision will be such that these procedures will be carried out in a humane and a scientifically acceptable manner as described herein. I understand that, as the research supervisor, 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 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, and I have marked all boxes pertaining to the relevant laws (and state permits) governing the species used in my research. I certify that my research will be conducted in accordance with all relevant federal and state laws.

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

11/25/2016

Date

Routing Slip for IACUC Protocol Reviews

Please keep this routing slip with the IACUC protocol you are reviewing. Please note, per our PHS Assurance, that reviews take place simultaneously on the same version of the protocol. Reviewers should communicate via phone or email to discuss any changes or concerns with the protocol.

Protocol No. 15 / 16 . W . 96 - A

Reviewer	Approve	Disapprove (Attach comments)	Date
Reiss (pt)	✓		12/6/16
Ward	✓		

Rick Z to
determine if
Protocol requires
vet signature

1st Review 11/14/16 RM
 2nd Review 12/5/2016 RM
 3rd Review _____
 4th Review _____