

**HUMBOLDT STATE UNIVERSITY**  
**INSTITUTIONAL ANIMAL CARE AND USE**  
**PROTOCOL ROUTING SLIP**

The attached protocol for the humane care and use of live vertebrate animals was submitted on

\_\_\_\_\_ by Richard Golightly for \_\_\_\_\_  
 (date) (faculty project leader) (course # if appropriate)

Check whether the work described in this protocol will be supported by funding administered by the (x)HSU Foundation, ( ) another administrative unit *-list* \_\_\_\_\_, or ( ) will be unfunded.

Animals used for this project will be housed in the following facilities (please check all that apply):

- ( ) Animal Rooms; ( ) Fish Hatchery; ( ) Game Pens; ( ) Telonicher Marine Lab;  
 ( ) Natural History Museum; ( ) Other, specify site and room \_\_\_\_\_

Person / phone number (or e-mail) to contact: Rick Golightly, 3952, rtg1

Project Title: Field testing of Conditioned Taste Aversion in Steller's Jays

♦ **ROUTE FIRST TO THE CHAIR OF THE IACUC**, ASSOCIATE DEAN OF THE COLLEGE OF NATURAL RESOURCES AND SCIENCES (RM. 106C IN THE FORESTRY BUILDING). 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.

**THE REMAINDER OF THIS PAGE IS FOR THE USE OF THE INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE**

Date 1 <sup>st</sup> Received _____	<b>REVIEW</b>	No. ____/____.____.____-____
<p>( ) 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 and the Campus Veterinarian.</p> <p>( ) 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, the Campus Veterinarian, and one additional member of the IACUC. Project topics will be reviewed by the IACUC at the next scheduled meeting.</p> <p>( ) 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.</p> <p>( ) 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.</p>		
_____ Signature, IACUC Member	_____ Date	( ) Approved ( ) Denied
_____ Signature, IACUC Member	_____ Date	( ) Approved ( ) Denied
_____ Signature, IACUC Chair	_____ Date	( ) Approved ( ) Denied
Routing slip revision 05/08		

cc: ( ) Project Leader, ( ) Animal Facility Supervisor, ( ) Department Chair

## PROTOCOL FOR THE HUMANE CARE AND USE OF LIVE VERTEBRATE ANIMALS

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.

Once completed, signed, and dated, please submit your protocols to the Chair of the IACUC, Associate Dean of the College of Natural Resources and Sciences, Forestry Bldg, Room 106C. All protocols should be submitted on the most recent version of the forms. For your convenience, protocol forms are available in several software formats from the Chair of the IACUC, from several department offices and stockrooms, and they can be downloaded from the IACUC web page (<http://www.humboldt.edu/~iacuc>). You can expedite the review process by following these formatting rules: avoid changing the format of the routing slip unless minor reformatting is necessary to keep it to a single page; leave an extra blank line between your answers and the questions; leave questions in bold-face type; type your answers in regular (non-bold) type; and format the final signature page so that it begins with the final question. Please contact the Campus Veterinarian, Dr. Richard Brown, (by phone-826-3320, or e-mail- RNB2@humboldt.edu) with questions concerning protocol preparation and submission.

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**1. Course Number** (if applicable).

**Project Title** (note that this title must match the title shown on the routing slip).

Field testing of Conditioned Taste Aversion in Steller's Jays

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**2. Responsible Faculty Member: Instructor, Principal Investigator or Project Director.**

**Name** Richard Golightly

**Department** Wildlife

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**3. Names of others involved in animal use activity and their qualifications to perform the procedures indicated.**

Pia Gabriel – PhD Candidate at HSU Wildlife Department  
Pia has been conducting field and laboratory research on Steller's Jays as part of her doctoral dissertation under the supervision of Prof. Jeff Black, HSU Wildlife Dept., since 2005. She has captured and banded over 500 jays in the course of her study and has extensive experience in handling, measuring, taking blood and behavioral testing in the field and laboratory.

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4. **Proposed starting date (the starting date cannot precede date of approval, and note that *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; annual review is automatic and you no longer need to submit an end date.

Date of approval

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5. **Scientific name, common name, and characteristics of all species to be used. List multiple species separately to explain variation in use. For field studies, please list all target species, species listed as protected, threatened, or endangered by the USFWS or the state in which the work will be conducted, and any non-target species that are likely to be impacted.**

Latin binomial	Common name	Sex	Age or Weight Range
<i>Primary target:</i>			
Cyanocitta stelleri	Steller's Jay	both	all
<i>Non-targets:</i>			
Corvus corax	Common Raven	both	all
Perisoreus canadensis	Gray Jay	both	all

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6. **Number of animals to be used. Explain why a smaller number would not allow you to meet your objectives (please provide clarification if based on statistical 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. Write N/A if this protocol covers only the transportation, use, and/or storage of carcasses or tissues.

Unknown: Over the range of the landscape somewhere between 150 – 350 jays may be exposed to the eggs, and probably less than 10 ravens. Reducing the study area and thus number of exposures would not allow statistical sensitivity and would subject the sample to localized effects, which would confound interpretations.

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7. **Source of the animals (or tissues) to be used or the 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.

Field work will take place in Redwood National Park, Humboldt County, California.

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8. **If live animals are to be maintained in captivity for greater than 12 hours, explain where and how the animals will be housed and who will be responsible for their daily care.** If no animals will be maintained in captivity, please clearly state that to be the case. Write N/A if this protocol covers only the transportation, use, and/or storage of carcasses or tissues.

No animals will be captured or held in captivity.

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**9. Provide a non-technical description of the proposed goals, general methods, and the educational or scientific objectives that the proposed use is designed to meet.**

Predation on nests of the threatened Marbled Murrelet appears to have dramatically increased in recent years, relative to historic levels, throughout the murrelet breeding range in Oregon and California. Steller's Jays are suspected to be responsible for the major proportion of egg predation on murrelet nests. The continuing decline of murrelet populations has occurred despite protection of most of their habitat. Reducing predation pressure of jays on murrelet nests is an urgent management goal for the recovery of murrelet populations in California.

The behavior of an individual predator can be modified to avoid a specific food item through a non-lethal process called conditioned taste aversion (CTA). The stable, territorial social structure and long life expectancy of Steller's Jays presents a good opportunity for behavioral training of these nest predators, so they avoid murrelet eggs in the future. Experiments on crows and other predators have shown the potential of CTA to reverse even robust food preferences and influence food selection for at least 8 months in free-ranging populations. In laboratory tests, we have established a safe aversive agent and the effectiveness of a retained aversion for 8 weeks or longer in Steller's jays (IACUC prot. # 09/10.W.07.B). We now propose field tests and development of a protocol for CTA with treated murrelet-like eggs for resident territorial jays to avoid murrelet eggs. An optimized CTA strategy is meant to be implemented as an emergency measure until more long-term strategies for lowering nest predation on murrelets are in place.

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**10. Provide a complete and detailed description of all procedures to be performed involving live vertebrate animals.** Your response should address the handling and restraint of non-anesthetized animals; deprivation of food or water for a period that is atypical for this species; use of chemical or biological agents; the drawing of blood; the use of anesthetics, analgesics, sedatives or tranquilizers; surgical procedures; exposure to radioactive materials, known carcinogens, or highly toxic substances; and any post-operative procedures. Write N/A if this protocol covers only the transportation, use, and/or storage of carcasses or tissues.

Carbachol (carbamyl choline chloride) is a widely available emetic that is water soluble, odourless and tasteless at doses known to be capable to produce CTA in other species. Cholinergic agonistic emetics such as Carbachol are toxic however, and repeated doses could impact both target and non-target species.

In laboratory tests, we identified doses of 32 mg and 24 mg of Carbachol per egg to safely and effectively induce avoidance of treated eggs, and extend avoidance of untreated eggs for a maximum time period. Since we found no difference in the effectiveness between the two doses, future experiments will be conducted using the lower dose of 24 mg to minimize exposure of both target and non-target animals to the toxin. Based on an anticipated mean body mass of 115g in our study population of Steller's jays, this equals 209 mg/kg body mass. We found no evidence of lasting ill effects of exposure to either dose of Carbachol; all birds that consumed Carbachol-laced egg were released back into the wild in good condition, and were subsequently resighted.

Succession of procedures:

1) Conditioned Taste Aversion (CTA) field treatment

Commencing 4 to 8 weeks prior to the start of the 2011 murrelet nesting season (April-August) we will broadcast 250 Carbachol-laced, murrelet-colored eggs and 250 untreated,

control-colored eggs throughout known nesting habitat of Marbled murrelets. We will select 250 trees over an area of approx. 500 ha, that provide platforms suitable for placing an egg on at least two separate branches located in the sub-canopy below 45'. This area is only a small fraction of murrelet nesting habitat in the study region, but large enough to contain several murrelet nests and a representative sample of landscapes used by varying densities of both jays and murrelets in the region. We expect that somewhere between 150 and 350 jays will be exposed to the treated eggs in this area. Eggs will not be placed in the high canopy that murrelets actually use for nesting. This will keep both climbers and jays away from actual murrelet nest sites. Since the jays will be trained to avoid eggs based on color, not on position in the tree, it is reasonable to assume that the CTA will transfer to real murrelet eggs high in the canopy. One platform per tree will receive a Carbachol-laced, murrelet-colored egg, another platform on the same tree will receive an untreated, control-colored egg. One week to ten days after broadcasting we will climb the trees again and assess predation rates on treated and control eggs. Eggs will be attached to the branches, so that they can be opened and consumed, but not moved or cached by jays or ravens. Thus, eggs will be unavailable to ground dwelling predators. Two other species of corvids known to occur in the study area (Gray jays and Common ravens) could incidentally predate Carbachol-treated eggs. Gray jays with an approximate mean body mass of 75g would be exposed to 320 mg/kg body mass if they ingested a whole egg. According to allometric scaling, this is closely equivalent to a dose of 32 mg Carbachol per egg for Steller's jays. Since no long-term adverse effects of this higher dose was observable on Steller's jays, we expect no long-term adverse effects of the equivalent allometric dose on Gray jays. Furthermore, most Steller's jays ingested only a small portion of the treated eggs, and Gray jays would probably do likewise, thus further reducing their exposure. We expect that exposure of Gray jays to Carbachol-laced eggs might induce a similarly effective aversion. Common ravens are much larger (~ 1kg) than Steller's jays. Whether consumption of Carbachol-laced eggs at the proposed dose would induce aversion is therefore uncertain, but exposure relative to body mass would be very small. We consider the likelihood of exposure to other non-target animals to be very low.

## 2) Effectiveness monitoring

At the start of the murrelet nesting season in April 2011 we will repeat the broadcasting scheme employed in phase 3 on the same set of trees. We will again assess predation rates on both treated and control eggs. The effectiveness of the aversion conditioning treatment in protecting murrelet-colored eggs from predation will be determined by comparing the ingestion rate of murrelet-colored eggs to control-colored eggs between the first and second egg broadcast.

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## 11. Will any of these procedures cause pain or distress (other than that necessitated by collection, injection, and otherwise mild, momentary discomforts)? If so, please explain. Write N/A if this protocol covers only the transportation, use, and/or storage of carcasses or tissues.

Ingestion of Carbachol-treated eggs is expected to cause temporary illness, possible vomiting, in the study subjects. This effect is intended to induce avoidance of eggs in the future. The experiment is designed to utilize doses of aversive agent that minimize the exposure of target and non-target animals to the agent and avoid long-term ill effects.

In the laboratory study we observed vomiting (in 10 out of 18 jays), subdued activity and beak wiping as a result of Carbachol ingestion. These behaviors lasted for minutes to several hours after ingestion of aversive agent, and resulted in no observable lasting ill effects.

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- 12. For researchers, explain how you determined that this protocol does not unnecessarily duplicate previously published observations or experiments (cite the type of literature searches as well as any other resources used). For instructors, explain the value of the lesson that merits using live animals. Write N/A if this protocol covers only the transportation, use, and/or storage of carcasses or tissues.**

Current literature on the use of conditioned taste aversion in wildlife management demonstrates the need to develop and test methods specific to the target species and ecological circumstances. Although strategies have been developed for several corvid species in specific circumstances, this is the first study addressing Steller's Jays, and a specific test across a larger landscape to develop CTA strategies needed to protect murrelet nests in Old Growth forests.

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- 13. Provide alternative procedures that were considered and rejected as well as a brief explanation of why the alternative procedures were rejected. Write N/A if this protocol covers only the transportation, use, and/or storage of carcasses or tissues.**

A possible management alternative would be the culling of territorial jays. However, this would upset the relatively stable territorial structure of a resident Steller's Jay population and likely result in a temporary influx of even larger densities of jays attempting to occupy the territorial vacancies. Lethal removal could be counter-productive by increasing the chance of incidental predation on murrelet nests.

Moreover, jays are native components of the ecosystem. Extermination of one native species to protect another native species raises ethical issues, especially in parks and reserves that have mandates to protect all natural resources.

Thus tests on CTA were devised to avoid lethal control. We expect CTA to be both more effective in reaching the management goals and more humane in treatment of the targeted predators.

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- 14. Identify serious human health risks (expected exposures to disease agents, toxic chemicals used, dangerous environmental conditions, etc.) to which any participants might be exposed during the routine performance of the duties proposed herein, and describe steps taken to mitigate those risks.**

Carbachol will be handled in very small quantities that are negligible as a risk to human health. MSDS data cites Carbachol toxicity as 40mg/kg LD50 in rats. The amount handled at one time will routinely be 24mg injected per treated egg (Jays are highly unlikely to ingest an entire egg by themselves). Carbachol is used in human eye drops in a concentration of 3%. The concentration we propose to use injected into eggs is 0.05%, or 1.6% of the concentration routinely used in human drugs.

Michelle Dostal has been consulting and training technicians on appropriate handling of Carbachol, as is the standard practice in our lab for all drugs or chemicals. Purchasing is coordinated with Michelle Dostal and Anthony Baker at the Biology Genetic Core Facility.

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**15. Describe the fate of the animals upon completion of the protocol. Include the procedure for euthanasia (if chemical, include drug, route, and dosage) and the method of verification (whether necessary as an experimental termination or in the case of unanticipated, accidental injury).** Note (1) that you must justify the scientific necessity for any variations from the established guidelines for euthanasia ([2000 Report of the AVMA Panel on Euthanasia](#) as published in the Journal of the American Veterinary Medical Association, 2001, 218(5): 669-696 or its replacement in the Code of Federal Regulations), (2) that you must report unexpected deaths to the IACUC as soon as possible to consider options, and (3) that you may write N/A only if this protocol covers only the transportation, use, and/or storage of carcasses or tissues.

For jays, cervical dislocation will be used should euthanasia be required in the field. Vital signs (breathing, pulse, eye movement) will be measured to confirm death.

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16. 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 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 \_\_\_\_\_

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\_\_\_\_\_  
Signature, Responsible Faculty Member      Date

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**Review by the IACUC Attending Veterinarian (if necessary):**

\_\_\_\_\_  
Signature, HSU Veterinarian      Date       Approved       Denied  
Explanation of denial:

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

\_\_\_\_\_  
Signature, IACUC Chair      Date       Approved       Denied  
Explanation of denial: